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Effects of Alcohol on *Trichinella spiralis*.*

G. N. Pierce, Jr., and James B. McNaught. (Introduced by William Dock.)

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Since there are reports that the liberal use of alcoholic beverages while ingesting trichinous meat offers protection against infection and since the use of generous quantities of cognac has been advised as therapy in trichinosis,[1] we have investigated this problem by experimentation on rats and by the following *in vitro* studies.

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* Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

[1] Lommel, F., *Handbuch der Inneren Medizin.* J. Springer, Berlin, 1925. Vol. 1, p. 1429.

Three white rats, infected 3 months previously with *Trichinella spiralis* obtained from a human diaphragm, were sacrificed, skinned, eviscerated, and passed through a meat chopper. The ground meat was thoroughly mixed. The following *in vitro* experiments were performed with this material.

Experiment No. I.—Effect of alcohol on digestion. Five gram quantities of the ground rat meat were placed in each of six 250 ml. glass beakers with 100 ml. of artificial gastric juice.[2] Water was added to the first 3 beakers in quantities of 10, 20, and 30 ml. while absolute ethyl alcohol in similar quantities was added to the remaining 3. All were stirred simultaneously in an incubator at 37°C. according to methods previously described.[2, 3] When examined after 6 hours the mixtures in the first 3 beakers were completely digested, while those in the last 3 containing the various dilutions of alcohol showed only partial digestion. All mixtures were permitted to digest for 18 hours when they were strained through a 60 mesh brass wire sieve into separatory funnels. Portions of undigested meat remained on the sieves of the 3 alcohol mixtures in amounts that increased with the strength of the alcohol. The larvae settled rapidly. The sediment of each was drawn off into separate graduated mixing cylinders and made up to 25 or 50 ml. volume with tap water. After thorough mixing, six 0.1 ml. quantities were pipetted into the depressions of hanging-drop slides where the larvae were readily counted using a magnification of 40 diameters. The total numbers of larvae in the concentrate from each 5 gm. of meat are shown in Table I. The 3 non-alcoholic digests averaged 12,732 living larvae while the number in the alcoholic digests varied from 7,660 to 2,650. In the 9% alcohol only 2% of the larvae were tightly coiled, the remainder were loosely coiled non-motile, pre-

TABLE I.
Showing Interference of Alcohol with Digestion and Liberation of Trichinella in 5 gm. Trichinous Meat.

Sample No.	Gastric Juice Ml.	Water Ml.	Absolute alcohol Ml.	Alcohol actual %	Number of Larvae Found		
					Living	Capsulated	Dead
1	100	10			13,150		
2	100	20			13,550		
3	100	30			11,500		
4	100		10	9.0			7,660
5	100		20	16.6		400	3,600
6	100		30	23.0		790	1,860

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[2] McNaught, James B., and Anderson, E. V., *J. Am. Med. Assn.*, 1936, **107**, 1446.

[3] Newman, H. W., DeLamater, J. N., and McNaught, J. B., *J. Lab. and Clin. Med.*, 1936, **22**, 203.

sumably dead. In the 16% strength approximately 10% of the larvae were still encapsulated while in the 23% fully 30% were encapsulated, some with fragments of muscle still adherent, and none of the liberated larvae were active.

It is obvious that alcohol increasingly interfered with the liberation of the larvae by digestion. This is in accordance with the experiments of Blotner,[4] who demonstrated that alcohol destroyed the proteolytic activity of certain gastrointestinal enzymes *in vitro* and *in vivo*. Others[5, 6] have shown that alcohol evokes a flow of gastric juice of high acidity but low digestive power.

Experiment No. II.—Direct action of alcohol on free trichinella larvae. Approximately 1000 living trichinella larvae digested free of their capsules were added to each of 10 small test tubes containing 2 ml. of aqueous solutions of ethyl alcohol ranging in strength from 25 to 0.1% in the first 9 tubes with water in the tenth as a control. The strength of alcohol was reduced by half in each tube, *i. e.*, tube 1 contained 25% alcohol, tube 2 contained 12.5%, etc. The tubes were kept in a 37.5°C. incubator except during observations.

The activity of the larvae were readily observed under a magnification of 40 diameters. Living larvae were in tight coils or fairly active. The dead ones assumed a comma shape. For 6 hours there were no changes in the larvae in any of the tubes. At 12 hours all larvae in tube 1 (25% alcohol) were dead. At 24 hours all were dead in tubes 2 and 3 (6.25%). By 48 hours all larvae were dead in the first 5 tubes (1.5%), while at least half were active in each of the last 5 tubes including the control. This type of an experiment was repeated 4 times during a 2 months' period, using trichinella from various rat digests with similar results.

These observations show that trichinella larvae are surprisingly tolerant to alcohol, resisting a concentration of 25% for more than 6 hours and of 12.5% for more than 12 hours. Neither of these concentrations are maintained in the human stomach for such long periods.

Conclusion. We conclude that a concentration of 25% alcohol has little direct action on trichinella *in vitro* but that concentrations as low as 9% interfere with the digestive liberation of encysted trichinella.

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[4] Blotner, H., *J. Am. Med. Assn.*, 1936, **106**, 1970.

[5] Krueger, L., and MacIntosh, F. A., *Am. J. Dig. Dis. and Nut.*, 1937, **4**, 104.

[6] Babkin, B. P., *Die äussere Sekretion der Verdauungsdrüsen.* 2nd Ed. 402-407, Springer, Berlin, 1928.

9316

Effect of Epinephrine on Blood Count and on Hematocrit Value.*

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The subcutaneous injection of epinephrine produces certain changes in the blood count.[1-4] These changes are not dependent upon the presence of the spleen.[5] The leukocytosis that follows injection of epinephrine has been attributed to splenic contraction, hemoconcentration, stimulation of the bone-marrow, redistribution of formed elements of the blood, expulsion of leukocytes from lymph-nodes, and stimulation of the vegetative nervous system. Recently it has been reported that injection of epinephrine produces a rise in the serum potassium[6-8] and that the action of potassium is responsible for the reactions ascribed to epinephrine.[9]

Human subjects were injected into the deltoid region with single doses of one cc. of a 1:1000 solution of epinephrine hydrochloride. The usual reactions of anxiety, palpitation, hyperidrosis, tremor and elevated blood-pressure occurred. Some of the patients who had diseases of the blood-forming tissues complained of soreness over the spleen and tenderness over the long bones.

In the first experiment all blood-counts were made from capillary blood (U. S. Bureau of Standards equipment). Differential blood-counts were made on cover-slip preparations stained by the Jenner-Giemsa method. Hemoglobin determinations, and erythrocyte, leukocyte, differential, and platelet counts were made before injection. Leukocyte and differential counts were made also 10, 20, 40, 60, and 100 minutes after injection.

In the second experiment the complete studies enumerated above, together with calculations of the mean corpuscular volume, were made on samples of venous blood withdrawn before injection and at

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* Assisted by a grant from the Christine Breon Fund for Medical Research.

[1] Loeper, M., and Crouzon, O., *Arch. d. Med. Exp.*, 1904, **16**, 83.

[2] Frey, W., and Lury, S., *Z. f. d. Ges. exp. Med.*, 1914, **2**, 50.

[3] Frey, W., and Hagemann, E., *Z. f. klin. Med.*, 1921, **92**, 450.

[4] Hatiegan, J., *Wiener klin. Wchnschr.*, 1917, **30**, 1541.

[5] Lucia, S. P., Leonard, M. E., and Falconer, E. H., *Am. J. Med. Sci.* In press.

[6] D'Silva, J. L., *J. Physiol.*, 1934, **82**, 393.

[7] D'Silva, J. L., *J. Physiol.*, 1936, **86**, 219.

[8] Schwarz, H., *Arch. f. exp. Path. u. Pharmacol.*, 1935, **177**, 628.

[9] Camp, W. J. R., and Higgins, J. A., *J. Pharm. and Exp. Therap.*, 1936, **57**, 376.

the five stipulated intervals after injections. The Wintrobe tube was used for hematocrit determinations, and each sample was spun to constant volume at about 2500 revolutions per minute for 3 or more hours.

Results. Experiment I: In all but 4 of 68 patients studied, the majority of whom had diseases of the blood-forming tissues, the injection of epinephrine was followed by a definite leukocytosis. The blood of 8 patients who had undergone splenectomy showed a leukocytic response similar to that of patients whose spleens had not been removed. In 56 cases, the increase in number of lymphocytes was earlier and relatively greater than that of the other cellular elements; whereas the percentage of polymorphonuclear neutrophiles decreased immediately after injection, and then gradually increased as the lymphocytes returned to normal values. In the remaining 12 cases there was no considerable change in the percentage of lymphocytes. Except in 2 cases (one of erythroblastic anemia and one of hemolytic icterus), immature cell-forms did not appear in the circulation in numbers sufficient to indicate stimulation of the bone-marrow.

Experiment II: In 10 patients studied, the injection of epinephrine was followed by an increase in the erythrocyte-counts. The maximum values were noted at 20 and 40 minutes after injection, following which there was a gradual decline to the pre-injection levels by the end of 100 minutes. The content of hemoglobin showed corresponding fluctuations. In all cases a leukocytosis occurred. The maximum leukocyte-count was noted at 10 minutes in 2 cases, at 20 minutes in 5 cases, and at 40 minutes in 3 cases. In all cases the percentage of lymphocytes first increased and then gradually decreased; whereas the percentage of polymorphonuclear neutrophiles decreased immediately after injection and then gradually increased as the lymphocytes returned to normal values. In all instances, the volume of packed cells increased after the injection of epinephrine and gradually returned to normal levels. The maximum hematocrit value was found at 10 minutes in 4 cases, at 20 minutes in 4 cases, and at 40 minutes in 2 cases. In 5 cases the mean corpuscular volume was found to be increased, the maximum values occurring 10 minutes after injection (maximum variation 22 cubic micra); in 3 cases the volume was decreased and gradually returned to the pre-injection level (maximum variation 28 cubic micra); and in 2 cases, the volume fluctuated within wide limits (maximum variation 34 cubic micra). Tests for fragility of erythrocytes failed to show any alterations following the injection of epinephrine.

In 5 cases, the addition of epinephrine to samples of blood removed before injection was without effect.

Summary. Injections of epinephrine were found to produce the following reactions in patients with and without diseases of the blood-forming tissues, from whom samples of capillary or venous blood were withdrawn: 1. Tenderness over the spleen and long bones occurred in some of the patients who had diseases of the blood-forming tissues. 2. There was an increase in the erythrocyte and leukocyte counts regardless of whether or not the spleen was present. 3. The lymphocytes were the first blood-cells to increase in numbers following injection. 4. Immature cell-forms did not appear in numbers sufficient to indicate stimulation of the bone-marrow. 5. The hematocrit values were increased. 6. The mean corpuscular volume varied too greatly to be of significance. (The calculation of the mean corpuscular volume is at best only an approximation.) 7. The fragility of erythrocytes was not altered. 8. When added *in vitro* to whole blood, epinephrine did not produce significant changes. The results of these experiments are compatible with the hypothesis that the leukocytosis following injection of epinephrine is due to hemo-concentration.

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Local Irritation from Sodium Bisulfite as Preservative in Epinephrine Solutions.*

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A large number of the epinephrine preparations on the market contain sodium bisulfite in a concentration of about 0.1%, to prevent the oxidation of the alkaloid. As far as we can determine, no studies have been made on the possible irritant properties of sodium bisulfite, so that its suitability for a therapeutic product does not seem to have been adequately established. We have, therefore, tested the material, to see whether irritant phenomena could be produced by this chemical, either in pure solution, or in the media in which it is used clinically in local anesthesia.

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Sodium bisulfite is an extremely acid salt, as shown by the measurements recorded in Table I, obtained with the DeEds glass electrode.[1]†

TABLE I.
Hydrogen Ion Concentrations of Sodium Bisulfite Solutions in Normal Saline and Local Anesthetic Solutions.

Concentration Sodium Bisulfite %	pH of $NaHSO_3$ dissolved in 0.9% NaCl	pH of $NaHSO_3$ dissolved in 2% Procaine HCl containing 1:25,000 Epinephrine HCl
0.5	1.82	2.13
0.1	2.12	2.83
0.02	2.66	3.59
0.004	3.62	5.50
0.0008	6.95	5.58

A test for possible irritation of mucous membranes was made by instilling 3 times a few drops of the bisulfite solution in the conjunctival sacs of rabbits. A 0.5% concentration in saline solution caused immediate reddening and chemosis with profuse lacrimation; 0.1% concentration produced the same changes to a lesser degree, while a 0.02% concentration produced only moderate hyperemia. Since 0.1% is the concentration of bisulfite usually present in epinephrine solutions, there is a definite possibility that it would cause local irritation of mucous surfaces in the conjunctiva, and possibly other areas as well.

Irritation in subcutaneous areas may be tested for by means of the colloidal dyes.[2] For such a test, 0.5 cc. of each bisulfite solution in Table I, together with controls of saline and procaine-epinephrine solutions, were injected subcutaneously in the previously shaved abdominal skin of a rabbit. Then 10 mg. per kg. of trypan blue dissolved in 6% dextrose solution was injected intravenously. The colloidal particles of trypan blue are too large to pass through the endothelium of normal blood vessels. However, in an irritated or damaged skin-area, the large dye molecules cannot be held back by the endothelium and so escape into the tissues, thus causing locally a deep blue stain which sharply marks out the area of increased vascular permeability. According to this test, irritation was absent after the control solutions and those containing 0.004% bisulfite or less. The stronger solutions caused definite vascular damage in the injected areas as shown by the appearance of a deep blue color locally.

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[1] Kahler, H., and DeEds, F., *J. Am. Chem. Soc.*, 1931, **53**, 2998; DeEds, F., *Science*, 1933, **78**, 557.

† We are indebted to Mr. C. W. Eddy for making the pH measurements.

[2] Tainter, M. L., and Hanzlik, P. J., *J. Pharm. Exp. Therap.*, 1924, **24**, 179; Spagnol, G., *Arch. Exp. Path. Pharm.*, 1928, **137**, 250.

A final test of possible local toxicity from the bisulfite was made by injecting the solutions in Table I both intracutaneously and subcutaneously in the skin of the forearms of all 3 authors, using aseptic technic. For the intracutaneous injections, 0.05 cc. was used and for the subcutaneous, 0.1 cc. The subcutaneous injections of the bisulfite in saline solution caused intense hyperemia at time of injection, followed by some induration. Over 48 hours were required for the skin areas to return completely to normal. The intensity of the effects was proportional to the concentration of the bisulfite, and was definite even with the lowest concentration used. The intracutaneous injections of the saline-bisulfite solutions were painful and caused hyperemia lasting over 2 hours with concentrations of the bisulfite of 0.02% or higher, but were painless and blanched when the bisulfite was dissolved in the procaine-epinephrine solution. The presence of epinephrine stopped the flow of blood through the injected area, so that signs of inflammation did not become visible, even though tissue damage may have occurred. There was no necrosis or sloughing in any of the skin-areas, although some discoloration persisted for several days. The factor of individual sensitivity was present, since the reactions were not equally great in all 3 persons.

When a patient is given an injection of a 1:1000 epinephrine solution containing sodium bisulfite, as preservative, the tissues may be exposed locally to 0.1% concentration of the bisulfite. When the epinephrine solution is added to procaine, a common practice in making local anesthetic solutions, the resulting bisulfite concentration may fall between 0.001%, if the end concentration of epinephrine is 1:100,000, and 0.007%, with epinephrine concentrations of 1:16,000, as are sometimes used. The results obtained by us show that such concentrations of sodium bisulfite are not entirely innocuous, but may produce demonstrable, though variable, injury to the tissues at the site of application. The reason that irritation has not been recognized heretofore is that the strong vasoconstriction of epinephrine obscures or inhibits the inflammatory reddening and swelling,[3] although it cannot prevent damage to the cells. There is a definite possibility, therefore, that the local inflammatory reactions commonly seen, after epinephrine vasoconstriction has passed off, may be due, at least in part, to the irritant effects of bisulfite. This being the case, search should be made for substitute anti-oxidants which would be free from the comparatively minor, but nevertheless definite, disadvantages of sodium bisulfite.

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[3] Tainter, M. L., *J. Pharm. Exp. Therap.*, 1926, **27**, 201; **33**, 129; Tainter, M. L., and Reichert, F. L., *Arch. Path.*, 1928, **6**, 872.

9318

Experimental Inoculations of Trichomonads from Man into the Prostate Gland of Rats.

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The purpose of these experiments was to determine the duration of experimental infections with the 3 human trichomonads in the prostate gland of the rat. According to certain recent workers *Trichomonas vaginalis* may occasionally be a causative agent of chronic prostatitis.

Although the incidence of trichomonad infection in the vagina of women is fairly high (10-69.9%),[1] positive reports of *T. vaginalis* in the prostate of men have been rare. Stuhler[2] found 16 positive cases out of a total of 32,000 prostate secretions; Riba and Perry[3] reported 2 cases. Nitschke[4] examined the urine and prostate fluid of 40 men suffering from non-specific urethritis and found 5 of them positive for *Trichomonas vaginalis*. Working in conjunction with Drs. A. M. Meads and T. I. Buckley of Oakland, California, we have been able to demonstrate one positive case of *T. vaginalis* out of a total of 100 patients examined. In all cases the expressed prostatic fluids were cultured in L.E.B. medium and were examined after each 2 to 4 transplants before being discarded as negative. This one positive case was a single man, age about 45 years, having a past history of gonococcal infection 15 years prior to this examination. There was no urethral discharge present, the gland was normal in size and quite tender on massage. Microscopic examination of the expressed prostatic fluid showed approximately 5% pus cells and numerous flagellates.

The combined effect of bacteria and protozoa probably accounts

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[1] Bland, P. B., Goldstein, L., and Wenrich, D. H., *J. A. M. A.*, 1931, **96**, 157.

[2] Stuhler, L. G., *Proc. Staff Meeting Mayo Clinic,* 1933, **8**, 221.

[3] Riba, L., and Perry E., *J. Urol.*, 1929, **22**, 563.

[4] Nitschke, P. H., *J. A. M. A.*, 1936, **107**, 12.

TABLE I.

Organism—*Trichomonas vaginalis.*

Total No. of rats used, 15. % positive, 66.66.

Rat No.	Previous Passage thru rat	Source of Material	Results	Hours in Gland
1	None	Culture	+	4
2	”	”	+	4
3	”	”	—	5
4	”	”	+	5
5	”	”	—	5
6	”	”	+	6
7	Once	Rat No. 6	—	6
8	None	Culture	+	24
9	”	”	+	24
10	Once	Rat No. 1	+	25
11	None	Culture	+	30
12	”	”	—	48
13	Twice	Rat No. 10	+	103
14	”	Rat No. 10	+	121
15	None	Culture	—	144

Organism—*Trichomonas buccalis.*

Total No. of rats used, 15. % positive, 46.66.

Rat No.	Previous Passage	Source of Material	Results	Hours in Gland
1	None	Culture	+	24
2	”	”	+	24
3	”	”	+	43
4	Once	Rat No. 2	+	48
5	None	Culture	+	49
6	Once	Rat No. 1	+	121
7	Twice	Rat No. 6	+	144
8	None	Culture	Animal died in	96 hrs.
9	Once	Rat No. 1	” ” ”	96 ”
10	Twice	Rat No. 4	” ” ”	24 ”
11	”	Rat No. 4	” ” ”	24 ”
12	”	Rat No. 4	” ” ”	24 ”
13	”	Rat No. 4	” ” ”	24 ”
14	”	Rat No. 4	” ” ”	24 ”
15	Once	Rat No. 3	” ” ”	120 ”

Organism—*Trichomonas hominis.*

Total No. of rats used, 17. % positive, 64.7.

Rat No.	Previous Passage	Source of Material	Results	Hours in Gland
1	None	Culture	+	4
2	”	”	+	24
3	”	”	+	38
4	Once	Rat No. 2	+	48
5	None	Culture	+	48
6	”	”	+	66
7	”	”	+	72
8	”	”	+	101
9	Once	Rat No. 6	+	102
10	Twice	Rat No. 4	+	118
11	None	Culture	—	121
12	Once	Rat No. 3	+	149
13	”	Rat No. 6	—	168
14	Twice	Rat No. 4	Animal died in	96 hrs.
15	Three	Rat No. 10	” ” ”	24 ”
16	”	Rat No. 10	” ” ”	24 ”
17	”	Rat No. 10	” ” ”	24 ”

for some cases of prostatic trouble. Karnaky[5] inoculated the bacteria accompanying *Trichomonas vaginalis* into the vaginal tract of women and failed to obtain a vaginitis. When the bacteria were mixed with *T. vaginalis* he obtained the usual clinical picture.

In our experimental procedure material was obtained from the vagina of women and cultured in L.E.B. medium. An opening into the abdominal cavity was made by midline incision and a total of 0.5 cc. of this Trichomonas-bacteria culture was injected into 2 lobes of the prostate gland. After varying intervals (shown in Table I), the gland was removed and placed in culture medium to determine the presence or absence of Trichomonas. Comparative studies using Trichomonas-bacteria cultures of *T. hominis* and *T. buccalis* were also made.

Eight of 15 rats were unable to survive inoculation with *T. buccalis* and 4 out of 17 died when cultures of *T. hominis* were used. There were no deaths in 15 rats inoculated with *T. vaginalis*. The maximum survival time of *T. vaginalis* which had not undergone previous animal passage was 30 hours; of *T. buccalis* 49 hours, and of *T. hominis* 101 hours. The maximum survival times of *T. vaginalis, T. buccalis* and *T. hominis* after previous animal passage were 121 hours, 144 hours and 149 hours respectively. The increased time which the organisms are able to remain viable in the gland may be due to an increased degree of pathogenicity of the protozoa, or of the bacteria, or both, since in some cases the glands showed induration; or it may be due to an increase in the adaptability of Trichomonas to the glandular tissue.

Summary. The 3 species of Trichomonas do not show any significant difference in survival time in the prostate gland if they have had previous animal passage. It is interesting to note that without previous passage *T. buccalis* and *T. vaginalis* closely resemble each other in their survival time. This is in accordance with the opinions of several workers who have pointed out the morphological similarities of these two species.

Although it obviously cannot be assumed that conditions in the prostate gland of the rat and of man are identical, these experiments do indicate that trichomonads can survive for some time in this type of tissue. Furthermore, by making cultures of the expressed prostatic fluid the incidence of *T. vaginalis* may prove to be greater than present statistics indicate.

The authors wish to express their appreciation to Doctor C. A. Kofoid for help and advice in these studies, and to Doctors A. M. Meads and T. I. Buckley for their coöperation in this problem.

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[5] Personal communication.

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Determination of Polarity in *Fucus* Eggs by Temperature Gradients.

E. W. Lowrance. (Introduced by D. M. Whitaker.)

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About 18 hours after fertilization the spherical egg of *Fucus furcatus,* reared in sea water in the dark at 15°C., develops a rhizoid protuberance at one point on its surface.[1] By the time it has appeared the primary developmental axis of the embryo is fixed. It has been shown by other workers that the point of rhizoid origin can be determined by exposing the egg to unilateral white light,[2-5] to electricity,[6] to the proximity of neighboring eggs,[3, 4, 7, 8] or to a pH gradient.[9] The results of this study show that temperature gradients are effective in determining the point of rhizoid origin and therefore the primary polarity of the embryo. A detailed account of experiments and results of this study will be published elsewhere.

The experiments were performed in a constant temperature room at 15°C. Eggs embedded in sea water-agar jelly* were reared between glass capillaries used as thermodes. These were placed parallel, one mm. apart. A cold, dilute glycerol solution circulated through one thermode. The other thermode inclosed a nichrome wire which produced a definite amount of heat when a measured current passed through it.

Gradients of salinity and osmotic pressure that might develop parallel to the temperature gradients were prevented by causing filtered sea water at room temperature to flow over the top of the sea-water agar in which the eggs were embedded.

Temperatures were measured directly with very small copperconstantan thermocouples and with a high sensitivity galvanometer. The thermocouples were embedded in the sea water-agar medium near the eggs.

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[1] Whitaker, D. M., *Biol. Bull.,* 1936, **70**, 100.

[2] Rosenvinge, M. L. K., *Rev. Gén. Bot.,* 1889, **1**, 53.

[3] Kniep, Hans, *Jahrb. wissensch. Bot.,* 1907, **44**, 635.

[4] Hurd, Annie M., *Bot. Gaz.,* 1920, **70**, 25.

[5] Whitaker, D. M., and Lowrance, E. W., *J. Cell. and Comp. Physiol.,* 1936, **7**, 417.

[6] Lund, E. J., *Bot. Gaz.,* 1923, **76**, 288.

[7] Whitaker, D. M., *Biol. Bull.,* 1931, **61**, 294.

[8] Whitaker, D. M., *J. Gen. Physiol.,* 1937, **20**, 491.

[9] Whitaker, D. M., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 472.

* This medium served to hold the eggs in place and to eliminate convection currents between the thermodes.

Eggs were grouped into 2 main classes: those with rhizoids originating on the sides toward the warm thermode and those with rhizoids originating on the sides toward the cold thermode.

As a control, 225 eggs were reared at 15°C., embedded in sea water-agar, but with the temperature gradient omitted. Of these, 111 (49%) formed rhizoids on the sides toward the thermode which inclosed the unheated nichrome wire. This approximates 50% and indicates randomness. Eighty-two eggs were reared at 14.0° to 15.9°C., and were exposed to temperature gradients of 0.6° to 0.8°C. per 75 microns† for 24 hours beginning 2 hours after fertilization. The rhizoids of all (100%) of these eggs originated on the warmed sides. A photomicrograph of the results of a typical experiment is shown in Fig. 1.

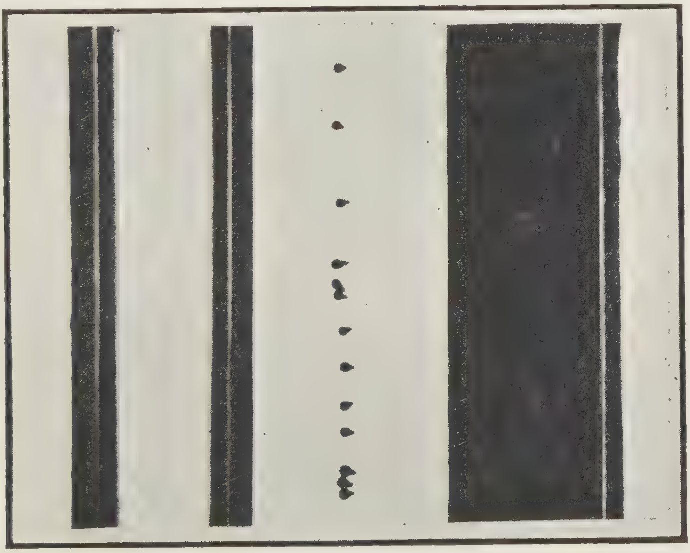

FIG. 1.

Eggs in sea water-agar developing in temperature gradient of 0.8° C. per 75 microns. Warm thermode is shown on right. Rhizoids originate on warmed sides of eggs.

The writer is indebted to Dr. D. M. Whitaker, under whom the study was carried out, for valuable advice and criticism.

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† 75 microns is the mean diameter of *Fucus furcatus* eggs used.

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Effects of Surgical Antiseptics on Streptofibrinolysin.*

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To determine the effects of surgical antiseptics on the fibrinolytic activity of *Streptococcus hemolyticus* tests were made of: (a) the effects of sub-bacteriostatic concentrations of the commoner antiseptics on the rate of fibrinolysin-production by glucose veal-infusion broth cultures of *S. hemolyticus*, and (b) the effects of certain selected concentrations of the same antiseptics on the coagulation and autolysis of human fibrin and on the liquefaction of isolated human fibrin by streptococcal cultures (or filtrates).

To determine the effects of antiseptics on fibrinolysin-production sub-bacteriostatic dilutions of the commoner antiseptics (Table I) were made in routine dextrose veal-infusion broth. Ten cc. of each of the resulting antiseptic-broth mixtures were inoculated with one loopful of a 24-hour broth culture of *S. hemolyticus* and incubated at 37°C. for 24 hours. The total bacterial population and the total number of fibrinolytic units per cc. were then determined for each subculture. (For technics see previous paper.[1]) Typical data are recorded in Fig. 1.

The table shows a partial inhibition of lysin-production in sub-bacteriostatic concentrations of zonite, the lysin-production varying proportionally with the rate of streptococcal proliferation. The same parallelism between bacterial count and lytic titer was noted with all other antiseptics (Table I) thus far tested by us. This parallelism tends to confirm our previous conclusion that there is a quantitative linkage between the rate of cell-division and the rate of fibrinolysin-production with *S. hemolyticus*.

To study the effects of the same antiseptics on coagulation and autolysis of human fibrin, solutions of fibrinogen and thrombin were prepared by the technic of Tillett and Garner.[2] Serial dilutions of the stock antiseptic solutions were made in the standard fibrino-

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* Work supported in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

† Eli Lilly Research Associate in Bacteriology, Stanford University, California.

[1] Madison, R. R., and Taranik, Jeannette D., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 1.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

TABLE I.

Effects of Antiseptics on Coagulation and Autolysis of Human Fibrin on Its Susceptibility to Streptofibrinolysin.

The first column records the highest dilution of each stock-solution that prevents *in vitro* coagulation of human fibrinogen-thrombin complex. Blank (—), no inhibition of clotting even with a 1:1 dilution of the antiseptic solution.

The second column records the highest dilution of the same stock antiseptic that will prevent subsequent autolysis. Blank (—), no inhibition of autolysis, even with a 1:1 dilution of the stock antiseptic.

The third and fourth columns record the arbitrary dilutions tested for their possible denaturing effects on human fibrin, and the percentage susceptibility of the resulting antiseptic fibrin complex.

	Highest dilution		Effect on fibrinolysis	
Stock Solution of Antiseptic	Inhibiting clotting	Preventing autolysis	Critical dilution	Fibrin susceptibility %
0.8% NaCl-solution (control)	—	—	—	100
0.1% Zonite	1:30	1:300	1:300	100
2% Phenol	—	—	1:3	100
1% Mercuric chloride	1:30	—	1:300	100
1% Merthiolate	1:30	—	1:300	100
1% Mercurochrome	1:30	—	1:300	100
0.6% Potassium permanganate	1:30	—	1:300	100
0.5% Metaphen	1:3	—	1:30	100
Mercresin*	1:3	—	1:30	100
0.03% Phenyl mercuric nitrate	—	—	1:3	100
Mi 31*	1:3	—	1:30	100
0.5% Hexyl resorcinol	stock	—	1:30	100
1.22% Picric acid	1:30	—	1:300	100
100% Glycerin	—	1:3	1:30	100
0.1% Acriflavine	1:3	1:30	1:30	100
97% Ethyl alcohol	1:3	—	1:30	100
5.15% Boric acid	1:3	1:300	1:30	100
2% Tannic acid	1:300	—	1:3000	30
Sulpho-salicylic acid (sat. solu.)	1:300	—	1:3000	30
0.1% Gentian violet	1:3	1:30	1:30	30
5.25% Hypochlorite	1:300	—	1:3000	20
2% Lysol	1:3	—	1:30	19
Tincture iodine	1:300	—	1:3000	12.5
0.029% Iodine in water	1:3	—	1:30	12.5
40% Formaldehyde	1:3	1:30	1:30	3
2.5% Prontosil	—	—	1:3	100
10% Sulphonamide	1:30	—	1:300	100
10% Thromboplastin*	—	—	1:3	100

*Standard commercial product.

gen-solution and coagulation attempted by the addition of a routine dose of thrombin. The maximal dilution of each antiseptic that prevents coagulation by this technic is recorded in the first column of Table I.

Antiseptic-free human fibrin prepared by this technic usually undergoes spontaneous autolysis within 24 hours at 37.5°C. Several of the antiseptics prevented this autolysis. The maximal inhibiting titers for these antiseptics are recorded in the second column of Table I.

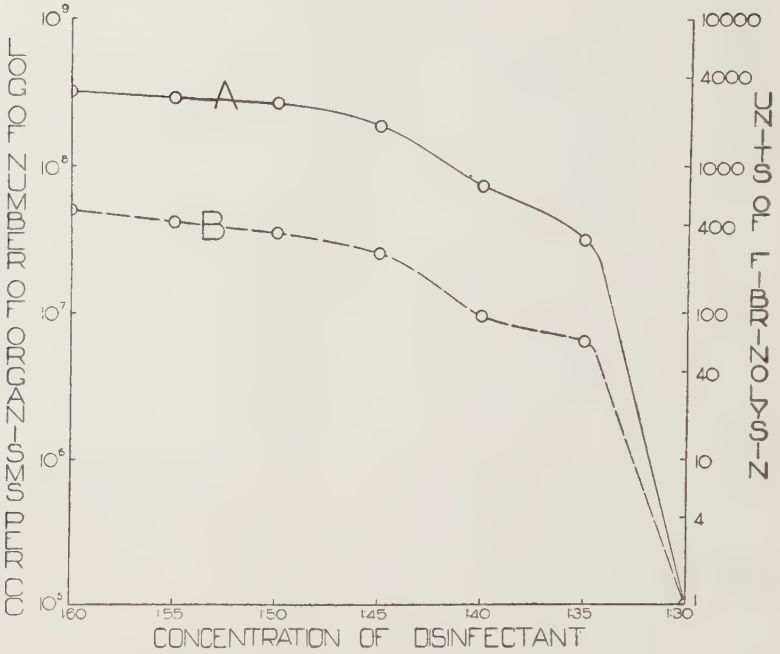

FIG. 1.

Typical Effects of Dilute Antiseptics on Streptococcal Proliferation and Fibrinolysin Secretion.

Data obtained with a 0.1% stock solution of zonite. 1:60 to 1:30 serial dilutions of this stock antiseptic in glucose, veal-infusion broth, inoculated with a standard loopful of a 24-hour broth culture of *S. hemolyticus,* and incubated at 37.5°C. for 24 hours.

A, Total number of streptococci per cc. in each 24-hour sub-culture, as determined by the Petroff-Hausser counting chamber.

B, Total number of fibrinolytic units per cc. in the same sub-cultures. (For a definition of the arbitrary fibrinolytic unit see previous papers.[1])

Possible chemical denaturation of human fibrin (or possible inhibition of the lytic enzyme) was tested with certain selected dilutions of each antiseptic. Data thus obtained are recorded as the relative susceptibility of the resulting fibrin-antiseptic complex to routine doses of streptofibrinolysin. Thus, if in a control tube of antiseptic-free human fibrin liquefaction is observed by the end of 2 hours as a result of the addition of a 1:500 dilution of a given streptococcal culture (or filtrate), while in the presence of the selected antiseptic, a 1:50 dilution of the same culture (or filtrate) is required, the antiseptic-fibrin complex is apparently but 10% as susceptible as normal human fibrin to streptofibrinolysin. Data thus calculated are recorded in the fourth column of Table I.

According to Menkin,[3] and others the main factor preventing the spread of staphylococci, streptococci and related microörganisms from a local focus is the meshwork of fibrin filling interstitial tissue-spaces and plugging regional lymphatics. If this is true, antiseptics which render encapsulating deposits of fibrin relatively insusceptible to streptofibrinolysins would be antiseptics of choice in the local treatment of streptococcal infections. In our hands tincture of iodine is more efficient in reducing fibrinolytic susceptibility than many of the newer antiseptics, a 1:3000 dilution of the tincture rendering fibrin but one-eighth its normal susceptibility to liquefaction by streptococci.

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[3] Menkin, V., *J. Exp. Med.*, 1931, **53**, 647; 1932, **56**, 157; 1933, **57**, 977.

9321 P

Ultrafiltration of Virus of Equine Encephalomyelitis (Russian Strain, Moscow No. 2)

A. S. LAZARUS* AND B. F. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco.

Bauer, Cox and Olitsky[1] have recently determined the filtration end-point of the Eastern and Western strains of the virus of equine encephalomyelitis. Their results have indicated a particle size of 20 to 30 mμ for both types. Previous work by one of us[2] has indicated the immunological independence of a Russian strain (Moscow No. 2) of this virus, and it was considered of interest to compare its size with the results already reported for the two North American strains.

A 20% suspension of guinea pig brain containing active virus was prepared in a diluting fluid consisting of equal parts of hormone broth, ascitic fluid and sterile distilled water as recommended by Bauer, Cox and Olitsky.[1] A 1-100 dilution of the stock suspension in the same fluid was used for the ultrafiltration experiments, while all titrations of the virus whether before or after filtration were regularly made in the same diluent.

The collodion membranes were prepared according to the method of Elford,[3] as modified by Bauer and Hughes.[4] All filtrations were conducted at room temperature under positive pressure of 20 to 50 cm. Hg. The filtrate collected varied from 2 to 4 cc., of which 0.03 cc. was injected intracranially into each of 3 mice for every membrane used.

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* Edith Claypole Memorial Research Fellow in Pathology, 1936-37.

[1] Bauer, J. H., Cox, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 378.

[2] Howitt, B. F., *J. Immun.*, 1935, **29**, 319.

[3] Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[4] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

Dilutions of 1-1,000, 1-10,000 and 1-100,000 of each filtrate were inoculated respectively into each of 2 mice as were similar dilutions of the unfiltered stock suspensions. The Russian strain gave a titer of 1-100,000 for the unfiltered material and 1-10,000 for the positive filtrates.

Comparative experiments with the Eastern (New Jersey) and Western (California) American strains were also made using similar dilutions for both filtered and unfiltered material. The Eastern virus filtrates were positive in a 1-10,000 dilution when passed through the finest membrane (average pore diameter 80 mμ) while the Western virus passed the same size membrane in a 1-100 dilution but was negative upon further titration.

The results of these studies show a sharp end-point for the Moscow No. 2 strain between membranes having average pore diameters of 160 and 180 mμ, and passage of the New Jersey and California strains through membranes having an average pore diameter of 80 mμ. Unfortunately, no membranes with smaller pores were available, and the exact end-point of the 2 American strains was not determined. However, these results supply partial confirmation to the findings of Bauer, Cox and Olitsky.[1] It is to be noted that the filtration end-point of the virus of Borna disease has been placed at 170 mμ,[5] the same figure obtained for our Russian strain of equine encephalomyelitis.

Summary. The Russian strain (Moscow No. 2) of equine encephalomyelitis virus has a particle size of 85 to 130 mμ, using the Elford type of graded collodion membranes and the Elford correction factor. Under the same filtration conditions, the California and New Jersey strains of the same virus have a particle size less than 40 mμ.

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[5] Elford, W. J., and Galloway, I., *Brit. J. Exp. Path.*, 1933, **14**, 196.

9322 P

The Bunsen-Roscoe Law Tested for Roentgen Rays on Mammalian Lymphoid Cells.

WILHELM STENSTROM AND JOSEPH T. KING.

From the Cancer Institute and the Department of Physiology, University of Minnesota.

A study has been made of the intensity factor in radiation using mammalian lymphoid cells in tissue culture as the test object. No previous data have been published using this material on the question involved.

Laser[1] found no difference in the growth rate of cultures of fibroblasts (avian) where the same dose was given at different rates. Canti[2] using cultures of chick fibroblasts found that higher intensities were more effective in suppressing mitotic rate. Cox[3] found the same effect on mitotic rate and also that the greater intensities and shorter time were more effective, judging by length of survival of cultures after radiation. Spear[4] studied both suppression of mitotic rate and length of survival in cultures of chick fibroblasts and found higher intensities more effective with the same dose. More recently, however, Faber[5] studying the growth rate of chick fibroblasts failed to find any difference when applying a given dose at different rates.

Fragments of the mesenteric lymph node of adult rabbits were planted in clots composed of one part heparinized rabbit plasma and 3 parts extract of 6-day chick embryos made with rabbit serum. This extract has been found to produce more vigorous, denser and more regular migration of lymphoid cells (King[6]).

Fragments were suspended in serum extract in carrel flasks and radiated before planting. This prevents the migration of cells into the clot before they have received the specified amount of radiation.

Fragments are radiated at 37.5°C.; control cultures are kept in the same thermostat protected with lead. Thirty-six cultures are prepared from one animal. These are arranged in 3 groups, control and 2 groups for radiation.

The maximal width of the migration zone at 24 hours is taken as index of the effect. The readings recorded are ocular micrometer units (114 = 1 mm.).

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[1] Laser, H., *Strahlentherapie*, 1930, **38**, 391.

[2] Canti, R. G., *Acta Radiol.*, 1929, **10**, 320.

[3] Cox, S. F., *Brit. J. Radiol.*, 1931, **4**, 111.

[4] Spear, F. G., *Brit. J. Radiol.*, 1931, **4**, 146.

[5] Faber, Borge., *Acta Radiol.*, 1935, Supplement xxi.

[6] King, Joseph T., *Am. J. Physiol.*, 1935, **113**, 81.

As a preliminary experiment, 2 series (30-36 cultures in a series) were run, giving the same dose in the same time to each experimental group in the series. Each group was radiated for 4 min. at 150 r/min. Radiation data: voltage 200 KV; current 30 Ma.; filter 2 mm. Al.; distance 50 cm. Half value layer 0.32 mm. cu.

The following results were obtained:

Control	4 Min.	4 Min.
149 (10)	100 (10)	101 (10)
147 (10)	101 (10)	98 (10)

Each figure represents the average of the cultures in the group. The number of cultures is given in parenthesis.

Four series were then run in which the same dose was given at 2 different rates. The rate was altered by changing the distance from 50 cm. to 119 cm.

The following results were obtained:

Control	4 Min.	22.7 Min.
162 (10)	101 (10)	103 (10)
154 (10)	99 (10)	101 (10)
136 (12)	82 (12)	79 (12)
148 (12)	93 (12)	81 (12)

This group of 4 series, when averaged, show values control 150; 4 min., 93.7; 22.7 min., 91.

Another group of 4 series were then run using a one mm. copper filter in addition to one mm. aluminum. The same distances were used, increasing the time to give the same dose.

Control	15.5 Min.	87.4 Min.
143 (10)	86 (10)	89 (10)
150 (10)	99 (10)	101 (10)
171 (10)	115 (10)	116 (10)
135 (10)	83 (10)	80 (10)

Averages for the 4 series—Control 149.7; 15.5 min. 95.7; 87.4 min. 96.5.

It should be noted that in a series (*i. e.,* cultures from a given animal) the migration rate may be faster or slower as compared with another series. When the radiated cultures are compared with their own controls the result is relatively constant.

When the 4 series in each group are averaged it is found on statistical analysis that there is no significant difference when the same dose is given at different rates. In a single series there was a difference of 12 units. The other series were all remarkably uniform.

Conclusion. Within the limits studied the Bunsen-Roscoe Law applied.

9323 P

Effect of Spaced Radiations on Lymphoid Cells in Tissue Culture.

JOSEPH T. KING AND WILHELM STENSTROM.

From the Department of Physiology and the Cancer Institute, University of Minnesota.

Spear[1] reported that the same delayed lethal effect was produced in cultures of fibroblasts by a 6-hour radiation (radium) whether given as one dose or as 6 one-hour doses on consecutive days.

He compared[2] the effect of single and spaced radiations (radium) using the depression of mitotic rate in cultures of fibroblasts as index of the effect. He found two radiations of 2½ minutes spaced 80 minutes apart more effective than a single 5-minute radiation.

He also found that dividing a 60-minute radiation into 24 doses of 2½ minutes given at intervals of 80 minutes greatly enhanced the effect. The spaced radiations were equivalent to a single dose of 4½ hours. In this study delayed lethal effect was the criterion used.

Faber[3] found that spacing a given dose of radiation did not alter its effectiveness in retarding the growth rate of fibroblast cultures.

In this study the migration rate of mammalian lymphoid cells in tissue cultures has been used to determine the effect of dividing a given dose of X-rays into 2 equal fractions with a time interval between. The extent of migration at 24 hours was used as index of the effect.

The culture technique is described in an accompanying paper.

Fragments of adult rabbit mesenteric lymph node were radiated suspended in serum chick embryo extract at 37.5°C. and subsequently planted. They were kept at this temperature during the interval between radiations. All cultures were kept in the radiation thermostat; those not being radiated were protected with lead.

Radiation data: 200 KV; 30 Ma; filter 2 mm. al; distance 50 cm. Half value layer 0.32 mm. copper.

The single dose was given in 4 minutes at either 150 or 184 r/min.; the split dose in 2 radiations of 2 minutes each with the designated interval.

Seven series (approximately 225 cultures) were studied in which the interval between the two 2-minute radiations was 3 hours. The results are given in tabular form.

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[1] Spear, F. G., *Proc. Roy. Soc. B.*, 1931, **108**, 190.

[2] Spear, F. G., *Proc. Roy. Soc. B.*, 1932, **110**, 224.

[3] Faber, Borge., *Acta Radiol.*, 1935, Supplement xxi.

TABLE I.

4 min.	2 min.-3 hr.-2 min.
108	104
103	103
101	95
96	92
93	89
90	79
95	84

The average for the single dose is 98; for the split dose 92.29. The average difference is 5.71; the standard error of the difference is 1.52.

In 3 series with a 1½-hour interval values of 102 for the single dose and 103 for the split dose were obtained.

In 2 series in which the 2-minute radiations were separated by simple stopping and starting the machine immediately, the value for both the single and split dose was 107.

Conclusion. Spaced radiation was found to be slightly more effective in inhibiting the migration rate of lymphoid cells when the interval between the radiations was 3 hours. Limited data at the indicated shorter intervals failed to reveal this difference.

9324 P

Origin of the Sympathetic Trunks in the Chick Embryo.

DAVID S. JONES. (Introduced by E. A. Boyden.)
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In most current textbooks of neuroanatomy and embryology the sympathetic trunks are described as arising from the spinal ganglia. This conception is based on the older descriptions and upon the more recent experimental investigations of Mueller and Ingvar[1] or Van Campenhout.[2] On the other hand, the sympathetic trunks are described as arising from the neural tube by a few of the earlier workers and by the majority of later workers, particularly by Kuntz.[3]

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[1] Mueller, E., and Ingvar, S., *Arch. f. mikr. Anat.*, 1923, **99**, 650.

[2] Van Campenhout, E., *Arch. de Biol.*, 1931, **42**, 479.

[3] Kuntz, A., *J. Comp. Neur.*, 1926, **40**, 389.

To gain further information on this subject 2 types of operations have been performed on chick embryos at 40-48 hours of incubation. First, the neural crests, with contiguous portion of the neural tube, were removed from the lower cervical and upper thoracic region for as many as 7 segments. The operation was performed under a binocular dissecting microscope by direct ablation with fine iridectomy scissors. The 25 embryos that survived this operation for a total incubation period of 7 days were then prepared for study by a modified Cajal technique, following which the nervous system in the region of operation was reconstructed graphically or in wax.

These 25 embryos may be divided into 2 groups, 13 of which (Group 1) have uninterrupted spinal cords extending throughout the region of operation and 12 of which (Group 2) have interrupted spinal cords, the gap ranging from one to 7 segments. The embryos of the first group show that the removal of neural crests and contiguous areas of the neural tube for as many as 7 segments does not prevent the formation of sympathetic trunks even though the spinal ganglia fail to develop in the area of operation. The embryos of the second group show that in cases where one to 3 segments of the spinal cord are missing (7 specimens) the sympathetic trunks will bridge the gap, but in cases where 2 to 7 segments of the cord are absent (5 specimens) the sympathetic trunks are interrupted. In the latter cases the sympathetic trunks extend into the region of operation for a distance of from one to 5 segments. Sympathetic ganglion cells however, migrate into the region only for a distance of from one to 3 segments. The migration of sympathetic trunks into the area of operation always proceeds from the cephalic end of the wound, never from the caudal end. Accordingly, if 3 segments is the maximum extent of migration of sympathetic neuroblasts, and this migration proceeds only from the cephalic end of the wound, it is apparent that the sympathetic trunks of an embryo in which 7 consecutive spinal ganglia are missing could not have resulted from migration of cells from the ends of the wound. So it must be concluded that the trunks arise from cells of the neural tube that migrate along the pathway of the ventral roots and communicating rami.

Further evidence that this conclusion is correct is afforded by a study of the normal 3-day chick embryo. At this stage the ventral roots of the spinal nerves are crowded with large neuroblasts similar in morphology to those in the wall of the neural tube. A continuous cellular strand of neuroblasts may be followed from the neural tube along the ventral roots and communicating rami to the position of the sympathetic trunks dorso-lateral to the aorta.

The negative results of Mueller and Ingvar[1] who found that destruction of the neural crests in the 48-hour chick would prevent the formation of the sympathetic trunks are explained on the ground that in using an electric cautery, the effects of which cannot be localized, these investigators injured more than the neural crests and so delayed or prevented the outgrowth of neuroblasts from the ventral portion of the neural tube. Also, the significance attached to the occurrence of both spinal and sympathetic ganglia in amyelous monsters without ventral roots is discounted by a 7-day embryo with an unclosed neural tube, but with ventral roots, spinal and sympathetic ganglia—an early stage of what would undoubtedly have become an amyelous embryo.

The weight of evidence afforded by these experiments and considerations, therefore, seems to favor the view that the source of most, if not all, of the neuroblasts that form the sympathetic trunks, is the neural tube.

9325

Production of Canine Blacktongue on Purified Diets.

HAROLD R. STREET. (Introduced by F. H. Scott.)

From the Laboratory of Physiological Chemistry, University of Minnesota.

Goldberger and coworkers[1] produced the canine disease blacktongue, on diets consisting principally of natural foodstuffs (largely corn, cow peas, and casein). They believed that the ability of certain foodstuffs to prevent the disease was due to their content of a specific heat-stable component of the vitamin B complex identical with the human pellagra-preventive (P-P) factor. The P-P factor has been rather generally considered[2] to be identical with the rat factor, vitamin G (B_2).

Certain doubts have been cast on this view of the production of blacktongue. Zimmerman and Burack[3] maintained dogs on an "artificial, balanced ration adequate in all dietary essentials as far as is known except water-soluble, heat-stable vitamin B_2 (G)," and these dogs did not develop blacktongue but a different disease.

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[1] Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *U. S. Public Health Reports*, 1926, **41**, 297.

[2] Aykroyd, W. R., and Roscoe, M. H., *Biochem. J.*, 1929, **23**, 483.

[3] Zimmerman, H. M., and Burack, E., *J. Exp. Med.*, 1934, **59**, 21.

Rhoads and Miller[4] also reported that they were unable to produce blacktongue by feeding diets lacking in G (B_2). It has even been suggested[3] that blacktongue is due to multiple deficiencies of the Goldberger diet rather than to lack of a specific vitamin.

The demonstration of the multiple nature of the heat-stable fraction of the B complex has led to an explanation of some of these discrepancies. Birch, György, and Harris[5] fed 4 dogs on a "synthetic" basal ration, complete except for the B complex, supplemented with lactoflavin [now known to be the rat factor, vitamin G (B_2)[6]] and highly purified vitamin B (B_1). Typical blacktongue appeared in each dog, proving conclusively that blacktongue is due to lack of some member of the vitamin B complex other than B (B_1) or G (B_2).

At the time of the appearance of the above work I had nearly completed an independent investigation on the production of blacktongue on "synthetic" diets. Four dogs were fed the following basal ration which is complete except for B vitamins: purified casein 19 parts, glucose 66, salt mixture (Osborne-Mendel) 4, cottonseed oil 8, and cod liver oil 3 parts. This was supplemented by a vitamin B (B_1) concentrate prepared according to the method of Evans and Lepkovsky.[7] Typical oral symptoms of blacktongue ranging from slight to acute occurred in the 4 dogs in 21, 21, 32, and 45 days, respectively. Control dogs receiving the same diet, with 6% autoclaved yeast added, remained perfectly healthy. These results are in complete agreement with those of Birch, György, and Harris.

In view of the complexity and controversial nature of this problem of the etiology of canine blacktongue, and the belief on the part of many investigators that the solution of this question can throw considerable light on the cause of human pellagra, this independent confirmation of the results of Birch, György, and Harris seems significant. From the work accomplished thus far there is little question that blacktongue involves in its etiology a lack of some water-soluble dietary factor or factors found in the so-called vitamin B complex and distinct from vitamins B (B_1) and G (B_2).

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[4] Rhoads, C. P., and Miller, D. K., *Science*, 1935, **81**, 159.

[5] Birch, T. W., György, P., and Harris, L. J., *Biochem. J.*, 1935, **29**, 2830.

[6] Booher, L. E., *J. Biol. Chem.*, 1934, **107**, 591.

[7] Evans, H. M., and Lepkovsky, S., *J. Nutrition*, 1931, **3**, 353.

9326 P

Experimental Exophthalmos in the Guinea Pig.

Donald L. Paulson. (Introduced by C. M. Jackson.)

From the Department of Anatomy, University of Minnesota.

Smelser[1] described a stainable infiltrate occurring between the fat cells, muscle fibers, the lobules of the lacrimal gland, and in the connective tissue of the orbit in thyroidectomized guinea pigs injected with thyrotropic hormone. This infiltrate was said to consist of granules and droplets, presumably lipoid, as well as many lymphocytes scattered throughout the tissue. He reported that human biopsy specimens of muscles, orbital connective tissue and fat obtained at operation from 2 low B. M. R. exophthalmos cases showed an infiltration indistinguishable from that obtained experimentally.

In the present study 12 immature guinea pigs whose weights ranged between 232 and 320 gm. were used. In 6 animals a thyroidectomy was done. One intact and one thyroidectomized animal were used as controls. Five intact and 5 thyroidectomized animals were given daily intraperitoneal injections of 25 to 40 guinea pig units of Antuitrin "T",* a potent anterior pituitary gland extract containing the thyrotropic factor separated from the other hormones of the hypophysis. Biopsy of the thyroid gland in 2 instances after 5 injections of the extract revealed a marked hyperplasia. The animals were sacrificed at intervals, after from 10 to 55 daily injections of the hormone. The control animals were sacrificed after 55 days, at the end of the experiment. At autopsy, the orbital contents were removed as one piece of tissue and specimens of skeletal muscle from various parts of the body were taken.

Exophthalmos was judged as having developed in 4 of 5 intact and in 4 of 5 thyroidectomized animals from the seventh to the tenth days of injection. The apparent proptosis was more definite early in the experiment and appeared to be of the same degree in both sets of animals.

Histologically, definite changes in the lacrimal gland were found in all animals injected with the thyrotropic hormone. While the lacrimal glands of the control animals occasionally contain isolated areas of excessive vacuolization of the alveolar cells, this is marked in the animals given thyrotropic hormone. The alveoli are abnormally dilated, and there are numerous large areas of apparent degeneration. The glandular cells contain large vacuoles and pyknotic nuclei. In some cells the cytoplasm is entirely gone. The connective tissue stroma is apparently increased and fibroblasts are present. In some areas this connective tissue preserves the outlines of the alveoli; in others the architecture of the gland is completely lacking. Collections of lymphocytes are only occasionally seen. These changes are most marked in the Harderian lacrimal gland (the extensive gland occupying a large part of the orbit in the guinea pig), but are seen also to a lesser degree in the intraorbital lacrimal gland (occupying the posterior angle of the orbit).

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[1] Smelser, G. K., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 128.

* Antuitrin "T" was provided for this experiment by Dr. E. A. Sharp, Director, Department of Experimental Medicine, Parke, Davis and Co. Each cc. contains 50 guinea pig units of the thyrotropic factor.

These findings are evident in the lacrimal gland of one animal sacrificed after receiving 10 injections of the extract, and are most marked in an intact animal with exophthalmos sacrificed after receiving 18 injections. In all other instances, these changes are present but to less extent, despite the fact that these animals received from 30 to 55 injections of the hormone. This fact appears to be correlated with the observation that the exophthalmos was most marked early in the experiment.

With respect to the extra-ocular muscles, in some instances a definite degeneration and interstitial edema are present. The muscle fibers are broken at intervals, have a granular appearance, and a loss of striations and substance is evident. The fibers are widely separated, and the connective tissue is edematous but not increased in amount. These findings are not consistent in the extra-ocular muscles of all animals, nor are they consistent in those muscles of the same animal. They were observed in 4 animals, 2 of which were thyroidectomized, and were most marked in a thyroidectomized animal sacrificed after 47 injections of the hormone.

No changes were found in the other skeletal muscles except for the *orbicularis oculi,* where in a few instances degeneration of muscle fibers is evident.

Reese[2] described degeneration, fibrosis and lymphocytic collections in the lacrimal glands of 2 human cases of progressive exophthalmos. It is possible that in man and the guinea pig these changes are the result of hyperfunction of the gland.

The pathology in the extra-ocular muscles appears to be similar to that accompanying progressive exophthalmos in man described by Burch[3] and Naffziger.[4]

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[2] Reese, A., *Arch. Ophth.*, 1935, **13**, 855.

[3] Burch, F. E., *Minn. Med.*, 1929, **12**, 668.

[4] Naffziger, H. C., *Ann. Surg.*, 1931, **94**, 582.

9327 P

Production of Mucoid Dissociants of *B. Coli* by Thermal Variation.

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On agar-plate cultures of *B. coli* stored at 18-20°C. for one week, a mucoid outgrowth from several colonies was observed. Subcultures from this mucoid structure indicated that the mucoid characteristic could be maintained only if cultures were incubated at this temperature. Revis[1] noted that mucoid forms of *B. coli* obtained by storage of organisms in soil would retain this characteristic if incubated at 20°C. but reverted to the more typical form if incubated at 37°C. Smith's[2] strain of mucoid *B. coli* from bovine sources retained its mucoid consistency when stored at 4.5°C. if drying out did not take place.

Our mucoid organisms were physiologically stable. Stains revealed a zoöglœic structure similar to that reported by Revis.[1]

To determine the influence of temperature on the persistence of the mucoid characteristic, transfers were made from the mucoid

TABLE I.
Incubative Temperature in Centigrade and Maintenance of Mucoid Variant of *B. coli.*

Mean Temp.,°C.	Limits of Temp.	Strain of *B. coli* 2	3	4
4	3 - 4.5	0	0	0
8	6.5- 9.5	0	0	0
9.5	9 -11.5	0	0	0
10	5.5-10	++++	++++	++++
13	11 -15	++++	++++	++
14	13 -14	++++	++++	+++
15	14 -16	++++	++++	+++
16	14 -17.6	++++	+++	+++
17	16 -17.6	+++	+++	++
17.6	14 -20	+++	+++	++
19.5	19.5	+	++	+
21.5	17.6-21.5	+	+	+
25	25	+	+	+
29	29	+	+	+
37	37	—	—	—

0 = no growth.
+ = degree of mucoidness of growth.
— = heavy growth but no mucoid character.

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[1] Revis, C., *Centralbl. f. Bakt., II Abt.*, 1910, **26**, 161.

[2] Smith, T., *J. Exp. Med.*, 1928, **48**, 351.

growth of 3 strains of *B. coli* on 1% glucose-agar plates. These were incubated at various temperatures for a period of 5 days before the type of growth was recorded as in Table I.

The marked correlation between incubative temperature and mucoid growth of *B. coli* indicates that the thermal factor is important in the production of mucoid dissociants of this organism.

9328 P

Bacterial Nutrition of the California Mussel.

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The sea mussel, *Mytilus californianus,* like other lamellibranchs, derives its nourishment from the microörganisms and finely divided detritus which it swallows.[1] It has been suggested that bacteria may constitute a very important part of the food of such plankton- and detritus-feeders.[2, 3] *M. californianus* was selected for experimentation along this line because of its abundance in the vicinity, its convenient size and the ease with which it can be maintained in the laboratory.

Specimens measuring 6-9 cm. in length and weighing 20-50 gm. were scraped clean of organisms attached to their shells and placed singly in liter jars of aërated sea water in the dark. After 2 weeks' inanition a bacterial suspension was added to each jar. A red coccus and a spore-forming bacillus which were morphologically and culturally distinctive enough to be readily recognized were used. They were grown on nutrient agar in Blake bottles and washed in 2 changes of saline by centrifuging. The mussels removed most of the bacteria from suspension within 2 hours as indicated by the clearing of the water,[4] whereas in control jars lacking mussels the water remained turbid for several hours. Three hours after the addition of about 200 million bacteria per cc., plate counts revealed the presence of less than ten thousand bacteria per cc. in jars containing mussels. That many of these bacteria were actually ingested and not merely clumped or rejected as pseudo-feces[4] was proved by microscopic and cultural demonstration of bacteria in the stomachs, especially on the head of the style, of dissected mussels soon after receiving a bacterial meal.

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[1] Fox, D. L., *Bull. Scripps Inst. Oceanogr.,* Tech. Ser., 1936, **4**, 1.

[2] MacGinitie, G. E., *Science,* 1932, **76**, 490.

[3] Baier, C. R., *Arch. f. Hydrobiol.,* 1935, **29**, 183.

[4] Fox, D. L., Sverdrup, H. U., and Cunningham, J. P., *Biol. Bull.,* 1937, **73**.

The disappearance of bacteria from the digestive tract within 6 hours after the bacterial meal and their paucity in the animal's feces were regarded as evidence that the bacteria were digested by the mussels. Further proof was obtained by demonstrating the occurrence of digestive enzymes which lyse bacteria. The viscera, including the digestive diverticula, stomach, style and intestine of several large well-fed mussels were ground with sand and the resulting extract pressed through cheesecloth. A few drops of this extract were added to toluene-preserved suspensions of bacteria buffered at pH 7.0 in small test tubes and held at 25°C. After different intervals smears of the mixture were stained and examined microscopically. Although much of the enzyme was probably lost by this technic, there was enough to dissolve the bacteria rapidly. The test has been made on 18 different marine bacteria[5] which were lysed by the extract. There was little or no lysis in the controls in which the extract was inactivated by heat.

Finally, the assimilation of the ingested and digested bacteria was proved by other feeding experiments. Small mussels 2-4 cm. long, weighing 2-5 gm. each, were used because small ones would be expected to grow more rapidly than larger ones. After an initial quarantine and inanition period of 2 weeks they were placed in groups of 3 per liter of filtered sea water. The water was aërated in the dark at 14-15°C. Three times each week bacteria were added in sufficient numbers to give about 200 million per cc. Eight jars received the red coccus, 8 received the spore-forming bacillus and nothing was added to the 8 control jars. Twice each month the mussels were weighed. Individuals were identified by depositing a drop of colored sealing wax on the shell. Once a month the water was replaced by filtered sea water. After 9 months, 47 of the original 72 mussels were still alive, including 17 of the 24 fed the coccus, 21 of the 24 fed the bacillus, and 9 of the 24 controls. Some of the survivors gained weight and others lost but a statistical treatment of the data revealed that the coccus-fed mussels gained an average of 0.46 gm. each or 12.4%, the bacillus-fed mussels gained an average of 0.39 gm. or 9.7%, and the fasting controls lost an average of 0.21 gm. each or 6.8%. It is estimated that during the 9-month period the mussels received 24 gm. of bacteria each and about

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[5] ZoBell, C. E., and Feltham, C. B., *Bull. Scripps Inst. Oceanogr.*, Tech. Ser., 1934, **3**, 279.

2.4 gm. of solid material, since the bacteria contain about 90% water. Mussels have been maintained in this laboratory for 16 months on an exclusive diet of bacteria. Professor T. Kincaid[6] has maintained oysters for several months with nothing to eat except bacteria. The oysters appeared to develop normally and their glycogen-content increased.

To date a total of 30 different new and undescribed species of marine bacteria[5] have been filtered from prepared sea-water suspensions by mussels, ingested and probably digested. In general, bacteria washed free of their metabolites do not seem to injure mussels unless so many are added that their respiration vitiates the water. In the presence of appreciable concentrations of bacterial nutrients such as peptone, the products of bacterial metabolism are usually toxic for the mussels.[1] The peptone itself is not toxic as indicated by the fact that mussels survive for several days in peptone solutions at refrigerative temperatures which retard bacterial activity.*

Most of the bacteria fed to mussels were alive. Heat-killed bacteria were also ingested by the mussels but they were not tolerated in as large doses as the living ones. This is attributed to the vitiation of the water by other bacteria which are always associated with the mussels and which multiply at the expense of the organic matter from the heat-killed bacteria. Bacteria known to be pathogenic for animals have not been fed to the mussels.

9329 P

Augmentation of the Gonadotropic Hormone from the Pregnant Mare.*

ALLEN LEIN. (Introduced by Bennet M. Allen.)

From the Department of Zoology, University of California at Los Angeles.

Several investigators have described the so-called augmentation phenomenon, which has recently aroused considerable interest in its applications to the study of the gonadotropic hormone obtained from the blood and certain other tissues of the pregnant mare. Cole and

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[6] Personal communication, University of Washington, Seattle.

* The authors acknowledge the technical assistance of Catharine B. Feltham and the helpful advice of Dr. Denis L. Fox.

* The writer wishes to thank Prof. B. M. Allen and Mr. Boris Krichesky for their many helpful criticisms and suggestions.

Hart[1] have shown that pregnant mare serum, when combined with pituitary synergist, is able to augment the action of the latter substance. However, non-pregnant serum, having no gonadotropic activity when tested on immature rats, was also found to augment the pituitary synergist, which is known to have follicle-stimulating activity. It was thus believed that both pregnant and non-pregnant mare serum perhaps contain a luteinizing hormone which is effective only when combined with pituitary synergist. However, later work[2] confirmed the findings of Maxwell,[3] who reported that inert materials such as proteins and salts of heavy metals, when combined with pituitary extracts, produce this augmentation phenomenon. Thus, it appears likely that the contained serum proteins, rather than any hormone, are responsible for the augmenting action of the mare serum.

The present work was undertaken in order to determine further the possibility of augmenting mare gonadotropic hormone through the use of pituitary factors. The mare hormone used was not taken from the blood serum but from the endometrium where it occurs in its greatest concentration. The pituitary preparation used was an acetone-ammonia extract[4] of sheep hypophysis, and its activity indicated that it contained primarily the luteinizing principle. The test animal throughout this work was the 21-day-old immature female mouse. Single injections of the pregnant mare gonadotropic hormone were made and the animals autopsied 5 days later; the pituitary factor, however, was administered in 3 doses given at 48-hour intervals, and the animals were sacrificed 5 days after the first injection.

Injection of a given quantity (6 rat units) of pregnant mare hormone into test animals increased the weight of the ovaries so that their combined weight in each animal averaged 4.3 mg. The above mentioned pituitary extract, when administered alone, had no observed effect on the weight of the ovaries of the test mice. Since it has been demonstrated repeatedly that luteinizing hormone is ineffective in immature animals, these results would appear to be what we should expect. In order to determine whether or not this pituitary luteinizing principle would augment the action of the pregnant mare hormone, these 2 preparations were administered simultaneously. The average weight of the ovaries of animals thus treated was 9.0 mg. This represents an increase of 109% in ovary weight.

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[1] Cole, H. H., and Hart, G. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 370.

[2] Saunders, F. J., and Cole, H. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 505; Fevold, H. L., Hisaw, F. L., and Greep, R., *Am. J. Physiol.*, 1936, **117**, 68.

[3] Maxwell, L. C., *Am. J. Physiol.*, 1934, **110**, 458.

[4] Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.*, 1934, **55**, 167.

In order to test whether this was a hormonal action or was simply due to the presence of proteins, the sheep pituitary extract was inactivated by heating at 100°C. for 25 minutes. Using doses equal to those previously injected, the heated material was administered to test animals simultaneously with pregnant mare hormone, and it was found that the heated hypophyseal extract had lost its ability to augment the action of the pregnant mare hormone. These data tend to indicate that the augmentation obtained by using unheated pituitary extract is dependent upon hormone activity rather than upon the presence of inert proteins. However, since heating is known to produce profound chemical changes in proteins, further experiments, which would control this factor, seemed advisable. Therefore, an extract of muscle, which had the same concentration of proteins as that contained in the pituitary solution, was prepared, and this was injected into test mice simultaneously with pregnant mare hormone. Ovary weights indicated that the muscle extract is incapable of augmenting the gonadotropic material from the pregnant mare.

It is of interest to note that, although inert materials such as proteins and salts of heavy metals are able to augment the gonadotropic hormone from the pituitary gland, our data seem to indicate that these inert materials will not augment the endometrial gonadotropic hormone of the pregnant mare. This is in accord with the work of Saunders and Cole,[2] who state that the addition of $ZnSO_4$ to pregnant mare serum has no effect in augmenting the action of the latter substance. In order to explain the above phenomena adequately and correlate them with other recently published work, it is obvious that further information concerning the underlying mechanism of augmentation is essential. Experiments which may throw some light upon this problem are now being performed in this laboratory.

Summary. The action of the gonad-stimulating hormone obtained from the endometrium of the pregnant mare is augmented by pituitary extracts containing primarily the luteinizing hormone. This augmentation is not brought about by using inactivated pituitary material or extracts of muscle. Thus, augmentation obtained in this experiment is dependent on hormonal action and is not produced by the use of the inert materials tested.

9330

Influence of Spleen on Leucocyte Count of the Albino Rat.

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Numerous investigators[1-5] have found that following splenectomy in dogs, rabbits, and rats there is produced a leucocytosis of unusual severity, which lasts longer than the time required for a return to normal following other surgical procedures of similar intensity. Bonanno[4] demonstrated that benzol administered to normal and splenectomized rabbits induced a consequent leucopenia, but that the renewal of leucocytes was more rapid in the splenectomized animals than in the controls. Lee[5] showed a change toward normal from leucocytosis following the injection of spleen emulsion to splenectomized rabbits. MacDonald[1] observed that the return to normal of the leucocyte count of dogs was more rapid, if at least 1/10 of the spleen was left *in situ* during splenectomy. Liles[6] found that the usual post-splenectomy rise in blood-platelet count decreased following the transplantation of spleen tissue into splenectomized rabbits.

These investigations seem to show that the spleen exerts some sort of inhibitory influence upon blood leucocyte and thrombocyte formation or release, which function is lost by the organism, at least temporarily, following splenectomy.

It was our purpose to observe the changes in the leucocyte count, total and differential, in *Bartonella-free* albino rats: (1) following splenectomy, (2) following splenectomy of rats with spleen transplants, (3) following splenectomy of rats with muscle transplants, and (4) following a major surgical operation, partial hysterectomy.

To determine the picture obtained following splenectomy, the spleens were removed from a group of 24 male and female albino rats. It was found that the post-splenectomy rise in leucocyte count was consistently 3 to 4 times normal, due to a marked myeloid neutrophilia. Blood was obtained by clipping off a portion of tail including 3 or 4 caudal vertebrae, a procedure used in all cases.

Four female rats, litter mates to some of the splenectomized animals, were partially hysterectomized, a procedure which consisted of excision of a portion of the left cornua of the uterus between a point about one cm. below the abdominal ostium and the uterine bifurcation. The post-operative rise in leucocyte count was found to be 1½ to 2 times normal, due to a slight neutrophilia. The return to normal took place in less than a month, a marked contrast with the splenectomized rats which retained a count above normal for several months.

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[1] MacDonald, *Am. J. Surgery*, 1934, **23**, 514.

[2] Tramontano, V., *Pathologica*, 1932, **24**, 667.

[3] Godard, H., *J. Phys. et path. Gén.*, 1932, **30**, 640.

[4] Bonanno, A. M., *Pathologica*, 1934, **26**, 832.

[5] Lee, Y. C., *Trans. Jap. Path. Soc.*, 1932, **22**, 299.

[6] Liles, R. T., Proc. Soc. Exp. Biol. and Med., 1926, **23**, 489.

Early in December, 1936, 4 eight-month-old rats received spleen transplants from younger rats, each animal receiving one whole spleen. These homiografts were planted in 4 equal pieces into 4 pockets in the fascia just ventral to the rectus sheath of the abdominal wall. Four other litter mates received muscle transplants similarly administered. Counts in both these groups one month following transplantation showed no significant change over counts obtained immediately before. Both groups were splenectomized February 11, 1937, and counts of tail blood were taken at varying intervals for a month and a half. On April 5 the sites of spleen transplantation were explored, and of the 16 transplants given more than 4 months previously, 14 appeared to have taken, as evidenced by reddish-brown nodes that were found in the positions of the former transplants. These nodes were excised, fixed, sectioned, mounted, and stained with Delafield's haematoxylin and eosin. The sections were studied and from the appearance of their histological

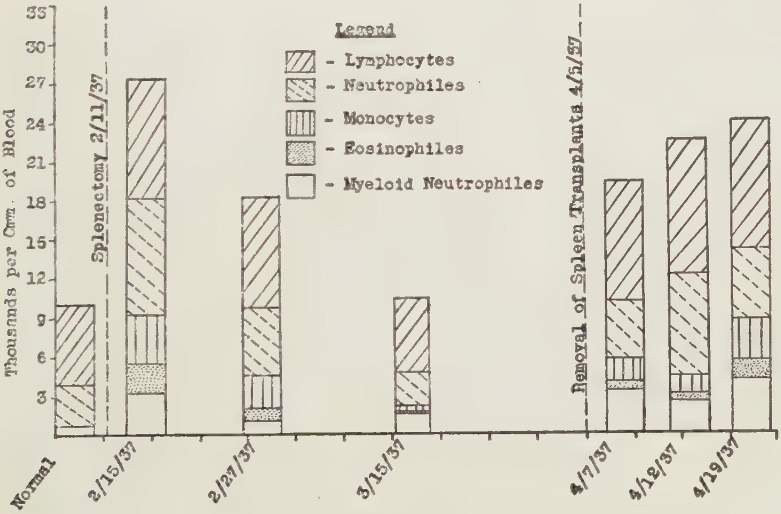

FIG. 1.

Leucocyte Count, Spleen Transplant and Splenectomy. Typical Case—Rat No. 62.

structure the spleen transplants were considered functional. After April 5, further counts were taken in each rat. (Fig. 1.)

Similarly, the muscle transplant sites were explored. As no particular centers of activity were found, 4 sections of fascia were removed from each of these animals on April 10, and blood counts made subsequently until April 19. (Fig. 2.)

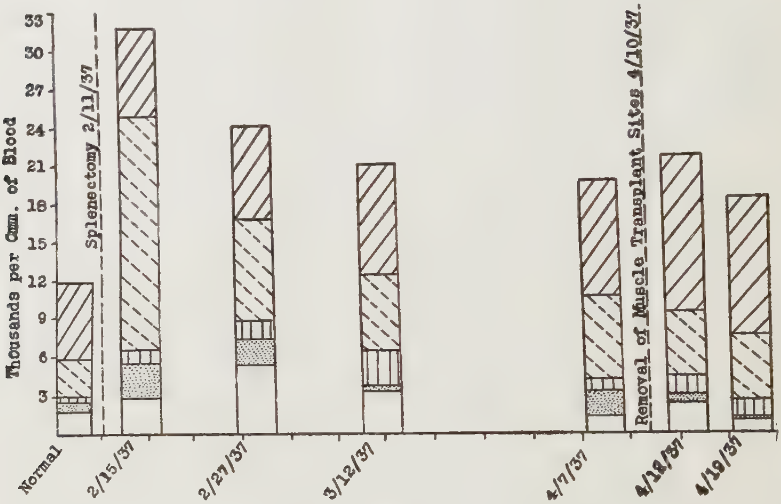

FIG. 2.

Leucocyte Count, Muscle Transplant and Splenectomy. Typical Case—Rat No. 69.

Summary. It is evident from the work described and the charts shown that in the *Bartonella-free* albino rat: 1. Following splenectomy, the total leucocyte count increases to 3 to 4 times normal. 2. This increase is due to a neutrophilia of a myeloid type which is more intense than that following other trauma. 3. Following a comparable surgical procedure, partial hysterectomy, the total leucocyte count increases to 1½ to 2 times normal. 4. This increase is due to a weak neutrophilia of a type that is usually found following surgical trauma. 5. Following spleen transplantation into a spleen-possessing rat, there appear to be no significant changes. 6. Following splenectomy, there is a lesser leucocytosis of a neutrophilic nature in the spleen transplant recipients than in the muscle transplant controls. 7. This leucocytosis approaches normal with greater rapidity in the spleen transplant recipients than in the controls. 8. Following removal of the spleen transplants, the blood picture resembles that following splenectomy, though in milder fashion. 9. Following removal of the muscle transplant sites, the blood picture indicates mild trauma. 10. Spleen homiotransplants may be 88% successful.

Conclusions. It is concluded that in the *Bartonella-free* albino rat: 1. Splenectomy induces greater neutrophilic leucocytosis than does a comparable surgical trauma, due to the consequent absence of an inhibitory influence on leucocyte production. 2. The normal spleen, as one of its functions, exerts an inhibitory influence on the leucopoietic centers, thus balancing the activity of leucopoietic-stimulating centers. 3. Spleen transplants function similarly to normal spleen tissue in controlling leucocyte production or release. 4. This inhibitory influence of the spleen on leucocyte formation or release may be considered as possibly an endocrine activity.

9331 P

Mechanism of Cellular Death by High Pressure, Compression of Yeast in Sodium Chloride Solutions.

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In a previous work[1] it has been shown that yeast cells suspended in tap water are killed in various lengths of time by pressures extending from 4000 to 7000 atmospheres. The present investigation was undertaken to determine the effect, if any, of the kinetic conditions resulting when high pressure is exerted on salt solutions of various concentrations.

The yeast, *Saccharomyces cerevisiae,* strain "Levure Royale", was obtained from the Anheuser-Busch Brewing Co. of St. Louis, in starch-free cakes. Two percent suspensions of the yeast in distilled water solutions of NaCl, varying in concentration from .005M to .4M, were put into glass vials of about one cc. capacity, stoppered with rubber corks. The pressure chamber consisted of a cylindrical cavity, 1.2 inch in diameter, bored in a steel block, and fitted with a hardened steel plunger. A rubber disc served as packing for the plunger. Four vials containing yeast in solutions of different concentrations could be compressed in the chamber at the same time. Care was taken to exclude air from the solutions in the vials and from the water which filled the chamber. A motor-driven hydraulic press supplied the pressure. From the readings of a previously tested gauge, mounted on the press, we calculated the effective pressures inside the chamber. After a 2-minute compression and a sudden decompression the yeast cells were stained with methylene blue and the number of dead (stained) cells was counted. The temperature of the room varied from 18° to 22°C.

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[1] Luyet, B., *Comptes Rendus de l'Ac. des Sc.*, 1937, **204**, 1506.

The results are plotted in the accompanying graph. Each point represents the percentage obtained in a count on 800 cells.

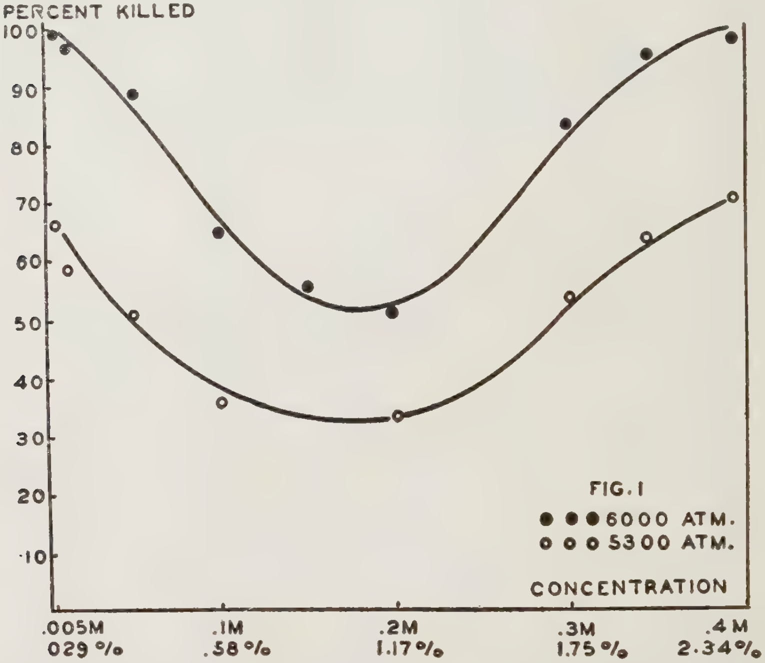

FIG. 1.
Number of cells killed at pressures of 5300 and 6000 atmospheres, in terms of the NaCl concentration.

In a previous investigation we found that pressures of 5300 and 6000 atmospheres applied to a tap water suspension of yeast, kills in 2 minutes respectively 48 and 76% of the cells. (Luyet, *op. cit.*). The addition of 3% NaCl to tap water increases the percentage killed at the same pressures to 69 and 94%. There is, therefore, a combined action of pressure and salt concentration. In the experiments with distilled water solutions reported in this paper, a similar combined action is shown at salt concentrations increasing from 0.2M to 0.4M (right hand side of the graph). But this combined action is not the sum of the injurious effects of each one of the 2 factors acting separately, for no yeast cell is killed by NaCl alone at the concentrations used. The kinetic conditions created by the reduction of the volume of the solution under pressure (about 13%

for water at 5000 atmospheres[2]) might explain the combined action of the 2 factors.

But the left hand side of the curves presents a different course and cannot be explained in the same manner. Here a decrease in the salt concentration increases the lethal action of pressure. The second of the 2 factors involved in the combined action appears to be the proportion of distilled water. We found that distilled water kills 50% of the cells in 90 minutes and that the addition of increasing quantities of NaCl makes it less and less injurious. So, taken separately, each one of the 2 factors is injurious and their combined action is more than the action of each factor alone. Whether or not it is equal to the sum of the 2 injurious influences cannot be decided at present and depends on the results of experiments now in progress in which the yeast is left in distilled water or in dilute solutions for various lengths of time before being pressed so that the degree of injury can be determined.

9332 P

Relation of Inclusion Blennorrhea to Swimming-Bath Conjunctivitis as Determined by an Accidental Transmission.*†

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That the conjunctivitis of infants designated as inclusion blennorrhea is transmissible to the adult was first demonstrated by Wolfrum[1] when he inoculated successfully 2 volunteers inducing what he considered to be trachoma. The diagnosis, however, based partially on the presence of inclusion bodies in the experimental disease and partially on his conviction of their specificity in trachoma, was not justified by the clinical manifestations. Gebb,[2] on the other hand, also reporting successful transmission, concluded the condition resulting from the inoculation was not trachoma. There the matter remained until recently when Thygeson[3] reported the results of his elaborately conducted and carefully controlled experiments in which material from infants with inclusion blennorrhea transferred to adults initiated not trachoma but swimming-bath conjunctivitis.

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[2] Bridgman, P. W., *The Physics of High Pressure,* London, 1931, p. 130.

* Conducted under a grant from the Commonwealth Fund of New York.

† This report is part of a more extensive study on the etiology of inclusion blennorrhea to be published later in the *American Journal of Ophthalmology* in collaboration with Drs. R. W. Harrison and A. C. Lange.

[1] Wolfrum, M., *Klin. monatsbl. Augenheilk.,* 1910, **48**, 154.

[2] Gebb, H., *Z. Augenheilk.,* 1914, **31**, 475.

During the course of experiments on inclusion blennorrhea in this laboratory, the opportunity was afforded to study the progress of an accidental transmission of this disease to an adult. While irrigating the eyes of an infant 11 days old, on the fifth day of suspected gonococcal conjunctivitis, some of the exudate was discharged into the left eye of the attending nurse. Despite immediate and vigorous lavage, a mild conjunctivitis was obvious on the following morning, when Dr. A. C. Lange was consulted. Because of the diagnosis in the case of the infant, optochine was prescribed, and on the fifth day the first appearance of small scattered follicles suggested a mistaken diagnosis in the case of the infant. Scrape smears of the conjunctiva stained by Wright or Gram showed neither inclusion bodies nor gonococci. On the sixth day, smears were repeated and numerous inclusions were found, but not gonococci. Smears made on the same day (11th) of the infant's conjunctiva likewise revealed inclusions without gonococci, thus justifying the diagnosis of inclusion blennorrhea. The infection in the nurse remained confined to the accidentally inoculated eye and progressed rapidly into swimming-bath conjunctivitis. The symptomology at the height of the infection consisted of a marked papillary hypertrophy of the conjunctiva which obscured the follicles to some extent, swelling and ptosis of the upper lid, and moderate discharge. Inclusion bodies were found at first with relative ease, and later in less and less numbers. After approximately 6 weeks during which grattage was performed twice , the infection subsided without either extending to the other eye or leaving any trace of its former presence, and inclusion bodies were no longer demonstrable.

Materials from both the infant and the nurse were inoculated in monkeys *(M. rhesus)*. Without desiring to enlarge upon the experimental results in the monkey, it is necessary to state at the present time only that there followed with both materials a follicular conjunctivitis, not only indistinguishable from each other but even from that of experimental trachoma. Of 8 monkeys inoculated, 6 were infected, and inclusion bodies were found in only 2. The inclusions numbered one in one instance and 2 in the other, and they were found only on one day early during the experimental disease.

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[3] Thygeson, P., *Am. J. Ophthal.*, 1934, **17**, 1019.

The conclusion seems warranted that inclusion blennorrhea and swimming-bath conjunctivitis are essentially the same disease, the former representing the conjunctival response of the infant, the latter the conjunctival response of the adult to the same etiological agent. Thus, the studies reported on this aspect of the disease by Thygeson receive confirmation from the present observation.

9333 P

Responses of the Kidney to Reflex and Direct Stimulation of the Splanchnic Nerve.

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The intravenous administration of epinephrine, as is well known, results in reduction of the kidney volume by reason of the vasoconstrictor action of the drug. Reversal of this reaction following the administration of ergotamine tartrate in small doses has been amply demonstrated.[1, 2] The experimental results here reported demonstrate the reactions of the kidney to reflex stimulation of its vasomotor nerves by means of other agents and to direct splanchnic stimulation both before and after the administration of ergotamine tartrate.

The experiments have been carried out on dogs under morphine-urethane and cats under nembutal anesthesia. Acetyl-beta methylcholine chloride was administered to the dogs by means of iontophoresis. In the cats all the drugs used were administered intravenously. In the experiments carried out on the dogs, the changes in kidney volume, blood pressure and urine output were recorded; in those carried out on the cats the records included changes in kidney volume, blood pressure and limb volume.

The typical effects of acetyl-beta methylcholine chloride administered by iontophoresis in dogs is a very short preliminary rise in kidney volume, followed by a fall corresponding to the fall in general blood pressure, and reduction in the urine output until after a few minutes it consists of an occasional drop. The duration of these

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[1] Hamet, R., *Compt. Rend. Acad. Sci.*, 1926, **182**, 1045.

[2] Wulp, G. A., and Nelson, E. E., *J. Pharm. Exp. Therap.*, 1931, **42**, 143.

experiments was about one hour. A current of 40 milliamperes was applied for 6 minutes. The output of urine is restored as soon as the blood pressure commences to rise toward the normal. When the blood pressure has returned to normal, the kidney volume increases until it rises above that recorded initially and returns to the initial level at the end of 5 to 10 minutes.

According to Hamet,[3] the initial rise in kidney volume and the secondary rise after the return of the systemic blood pressure to normal are both definite vasodilator responses of the kidney caused by acetylcholine. Acetyl-beta methylcholine chloride was used in these experiments because of its prolonged action and stability. Our results agree with those of Hamet, but if there is a definite vasodilator response to acetylcholine, there probably should be vasodilator fibers to the kidney vessels.

In the experiments carried out on cats similar results were obtained by the intravenous injection of 0.2 cc. of 1-1,000 solution of acetyl-beta methylcholine chloride. The initial rise in kidney volume was not evident, but there was a definite decrease in limb volume, subsequently followed by a slight increase. Kidney volume in the cats reacted in the same manner as it did in the dog.

Stimulation of the distal end of the cut splanchnic nerve causes a rise in blood pressure, a decrease of kidney volume and a rise in limb volume. After ergotamine tartrate the kidney response is reversed. One-half cubic centimeter of a 1-2000 solution of ergotamine tartrate was found to be sufficient to cause the reversal effect upon the kidney vessels but not upon the systemic blood pressure. This dosage corresponds to that found necessary by Hamet,[1] Wulp and Nelson,[2] and Van Dyke,[4] to elicit this reaction on the renal vessels. The minimum dosage necessary to cause the reversal effect on the renal vessels varies widely, from 0.0054 mg. to 0.11 mg. per kilo body weight, but is rarely as high as the latter. Stimulation of the splanchnic nerve after the administration of ergotamine causes a definite increase in kidney volume, but also a slight rise in systemic blood pressure and an increase in limb volume.

With the adrenal glands intact it is almost impossible to produce the reversal effect of epinephrine upon the blood pressure no matter how much ergotamine is injected; consequently, the right adrenal was ligated and the left either ligated or removed. Even after this has been done it is difficult to produce a reversal effect when the splanchnic nerve is stimulated at 12 cm. for 10 seconds.

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[3] Hamet, R., *Compt. Rend. Soc. de Biol.*, 1926, **94**, 727.

[4] Van Dyke, H. B., *J. Pharm. Exp. Therap.*, 1926, **27**, 299.

Our results indicate that by the time enough ergotamine has been administered to prevent the rise in blood pressure or to produce the blood pressure reversal effect by stimulation of the splanchnic nerve after ergotamine, the kidney vessels no longer react and are paralyzed so that stimulation produces only a small response which corresponds to the changes in blood pressure.

The reaction of the kidney vessels after small doses of ergotamine cannot be explained at present. Possibly the constrictor fibers to the kidney vessels are more sensitive to ergotamine than the other vessels, or their control over these vessels is less complete, consequently, they require less of this alkaloid to paralyze the endings, thus allowing the kidney vessels to react to adrenalin in the same manner that they do after section of the splanchnic nerve, *i. e.,* by dilatation.

The only direct response of the renal vessels seems to be constriction to adrenalin; all the other responses evidently are passive. The kidney volume increases or decreases as the blood pressure rises or falls.

The results of these experiments seem to indicate that the renal vessels are without vasodilator fibers and that the splanchno-peripheral balance probably plays the most important rôle in governing the vasomotor activities of the kidney and, consequently, kidney volume.

9334 P

Treatment of Serum Serving to Remove Its Serum-Sickness-Causing Activity.

L. R. JONES AND MOYER S. FLEISHER.

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We have reported previously concerning a reaction appearing on the ears of rabbits[1] subsequent to an injection of either whole horse serum or fractions thereof, which may be considered as analogous to serum-sickness in human beings.[2] In addition, studies concerning the serum-sickness-causing activity of the various serum proteins led to the suggestion that the more important substance concerned in causing serum-sickness may be some agent other than the proteins themselves although this agent appears to be associated with the pseudoglobulin.[3]

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[1] Fleisher, M. S., and Jones, L., *J. Exp. Med.*, 1931, **54**, 597.

[2] Fleisher, M. S., and Jones, L., *J. Immun.*, 1933, **24**, 383.

It has been found that by the addition of adequate quantities of alkali or acid to serum (normal or immune), exposure of the mixture to heat and subsequent neutralization, the serum-sickness-causing activity may be destroyed so that rabbits injected with the customary amounts of serum will not develop manifestations of the disease.

In order to minimize or prevent damage to antibody incident to this type of treatment of serum, it is necessary to adjust the various factors concerned in the treatment of the serum. Six factors must be considered: 1, The amount of alkali used (between 0.2 gm. and 0.1 gm. for 100 cc. of serum when using NaOH); 2, the dilution of the mixture (from 1:2 to 1:10); 3, the temperature to which the mixture is exposed (from 45°C. to 55°C.); 4, the time of exposure (from 30 min. to 18 hr.); 5, the type of antibody in the serum; 6, the particular sample of serum being treated. The first 4 factors must be balanced against each other in relation to the last 2 factors.

By this method the serum-sickness-causing factor may be destroyed so that rabbits receiving treated serum (10 cc. per kg.) do not develop serum-sickness while rabbits injected with the same untreated serum develop manifestations of serum-sickness in 6 of 10 animals. In sera which are no longer active in causing serum-sickness, from 50% to 100% of the original protective antibody is retained.

9335

Changes in Ribs of Guinea Pigs Following Administration of Cattle Anterior Pituitary Extract. (*Acromegalic Rosary*).

MARTIN SILBERBERG AND RUTH SILBERBERG.*

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In previous investigations we could show that after 10 to 21 injections of acid extract of cattle anterior pituitary a growth effect becomes manifest in cartilage and bone of young guinea pigs. The principal changes observed are as follows: (1) The epiphyseal cartilage in a first stage undergoes hypertrophy and hyperplasia; this is followed in the majority of cases by an accelerated calcification and ossification of the epiphyseal line.[1] (2) In certain cases hypertrophy and hyperplasia of the cartilage take place without acceleration of ossification. (3) Arthropathic lesions of an acromegalic type may occur in the joints.

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[3] Jones, L., and Fleisher, M. S., *J. Immun.*, 1934, **26**, 455.

* We are indebted to Dr. Leo Loeb, who made it possible for us to carry out these investigations.

These changes are accomplished without the mediation of the thyroid gland.[2]

In these investigations we wished to study the effects of the extract if administered over longer periods of time. The present report deals only with its action on the chondro-osseous junction of the ribs of normal as well as of thyroidectomized guinea pigs.

Twelve young guinea pigs of both sexes weighing on the average 140 gm. received daily intraperitoneal injections of 1 to 1½ cc. of acid extract of anterior pituitary of cattle for periods varying between one and 5 months, while 10 additional animals were not injected and served as controls. In 16 other guinea pigs of approximately the same weight the thyroids were completely removed under ether anesthesia. Eight of these animals received daily injections of acid extract for various periods, up to 5 months. The remaining 8 were not injected and served as controls. All the guinea pigs were kept under the same environmental conditions and their weights were taken daily. At the end of the experimental term various parts of the skeleton were fixed, incompletely decalcified, and stained, as described previously.

Gross appearances: In normal guinea pigs injected for longer than 3 months a thickening of the ribs in their entire length became noticeable. In addition a bulbous enlargement of the chondro-osseous junction of the ribs, particularly of the lower ones, was seen. In some instances small nodular newgrowths with a hard consistency and having a bluish white color were detected. These lesions were still more pronounced in the thyroidectomized injected animals. After 5 months' treatment a typical rosary-like condition of the chondro-osseous junction was found. The largest of these new formations were about the size of lentils.

Microscopic examination: The line of ossification is, on the whole, regular: The bonemarrow is sharply demarcated from the preëxisting cartilaginous parts. The changes occur in the zone of the resting and columnar cartilage as well as at the side of the inner layer of the perichondrium and the neighboring periosteum: The resting cartilage cells are more numerous than normally. Their nuclei are generally well preserved and their cytoplasm is somewhat enlarged; it takes on a light stain and contains small vacuoles here and there. The cells of the columnar cartilage are definitely hypertrophic, showing a markedly enlarged vacuolar cytoplasm. In some places the nuclei have assumed an intensely red eosin stain; distinct structural differentiations in the nuclei are lacking. A single cartilage-capsule may enclose as many as 4 or 5 cells and these cell groups may occasionally undergo degeneration, as is indicated by the liquefaction of their cytoplasm. The interstitial connective tissue is diminished in amount. Toward the periphery of the rib the cartilage is distinctly stained bluish by hematoxylin. The normally vertical arrangement of the columnar cartilage assumes more and more an oblique direction, until the position of the columns has become almost radial extending in a bow into the zone between periosteum and bone. In addition to the cartilage cells, the actively stimulated perichondrium and periosteum participate in the new formation by means of hypertrophy and hyperplasia of the spindle cells, and their growth activity may even exceed that of the cartilage proper. These spindle cells become more numerous and then assume a more oval and rounded shape; they are characterized by a large amount of cytoplasm, which encloses nuclei stained deeply blue with the hematoxylin; thus they resemble typical chondroblasts. In advanced stages the most centrally situated cells of the new growth appear polyhedral and are converted into chondrocytes. Here and there calcification of these cartilaginous cells is noticeable; the intercellular stroma is developed only to a very limited extent. In the most advanced cases the newly formed cartilaginous mass represents a callus-like structure situated between the periosteum and perichondrium on the one side and the original rib on the other side.

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[1] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423.

[2] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 66.

Associated with these changes are plastic processes within the bone-marrow, in particular in areas adjoining the cartilaginous growth. In the marrow, fibrillar connective tissue has replaced the lymphoid structure. Furthermore a conversion of these fibrous elements into cartilage cells could be observed.

In thyroidectomized animals, all these changes are even more marked than in the guinea pigs with intact thyroid glands. There exists, however, no essential difference between these two groups of animals.

Summary. Administration of acid extract of cattle anterior pituitary, when continued over long periods of time, leads to the following changes at the chondro-osseous junction of the ribs of the growing guinea pig: (1) The preëxisting resting and columnar cartilage cells undergo hyperplastic and hypertrophic changes which are associated with certain retrogressive processes in the cells. (2) In the cells of the perichondrium and periosteum a pronounced hyperplasia takes place followed by an accelerated conversion of these cells into cartilaginous tissue with subsequent calcification. (3) The bone marrow becomes transformed into fibrous connective tissue in which cartilaginous islands develop in places adjoining the hyperplastic cartilage. Through the coöperation of both these growth processes an abundance of cartilage may develop.

We find various transitions between ordinary hypertrophy and hyperplasia of the preëxisting cartilage cells to the formation of a rosary-like newgrowth. In advanced cases, at a time when usually the growth of the skeleton is terminated, the formation of tissue may become so very extensive that it leads to the production of a parosteal "rosary". These changes are very similar to those described by Erdheim[3] in cases of human acromegaly. They also correspond to the alterations observed by us in the cartilage of the epiphyseal line and the joint following injections of the extract over shorter periods of time. These changes may be even more marked in thyroidectomized than in non-thyroidectomized animals, which provides further proof for the conclusion that, in guinea pigs, cattle anterior pituitary extract acts on the growth of bone and cartilage without the mediation of the thyroid gland.

9336

The Fibro-Muscular Scrotal Sac Under Normal and Experimental Conditions in an Annual Breeder (*Citellus*).*

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What causes the testis of an adult ground squirrel to ascend into the peritoneal cavity during the non-breeding period? Is decrease in testis-size a primary factor, or does the scrotum regress and force the testis to ascend? The scrota of 89 ground squirrels *(Citellus*

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[3] Erdheim, J., *Veroeffentlichungen a. d. Gebiete d. Pathol. in Einzeldarstellungen,* Julius Springer, Berlin, 1931.

* Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

tridecemlineatus) have been studied in an attempt to answer these questions. Details concerning the normal sexual cycle in the male are presented elsewhere.[1, 2]

The present report deals largely with the muscular pouch which incloses testis and epididymis, designated in this paper by the name "fibro-muscular scrotal sac" (Fig. 2). This sac is composed of the following layers: peritoneum, internal spermatic fascia, cremasteric skeletal muscle and external spermatic fascia (Figs. 1, 3). The fibro-muscular sac of each animal was freed of its contents, severed at the inguinal ring (Fig. 3), weighed while fresh, fixed in Bouin's solution and sectioned at 6 micra for microscopical study.

The scrotal sac of prepuberal males undergoes a gradual increase in weight (Table I). The sacs of adults are large in males with large scrotal and spermatic testes, and are greatly reduced in size in animals having small, abdominal and aspermatic testes. Weight

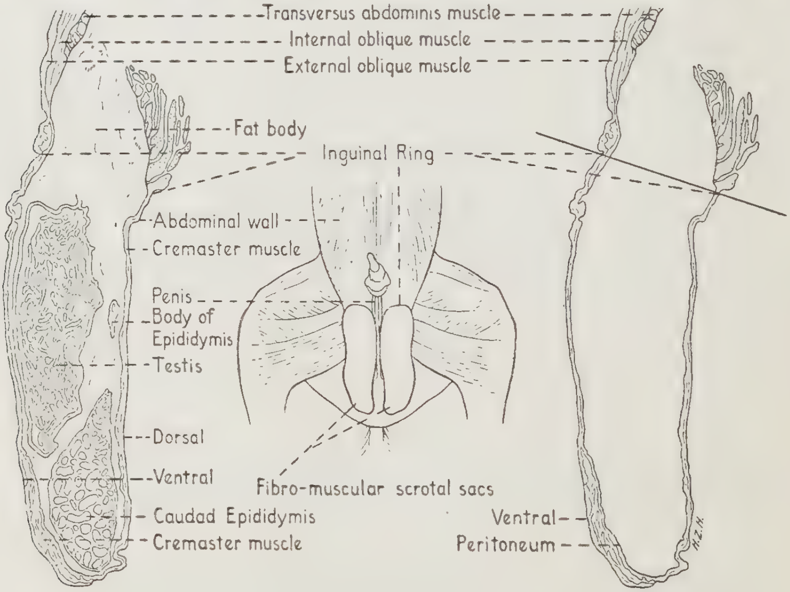

FIG. 1.

Longitudinal section through abdominal wall and fibro-muscular scrotal sac. Contents of sac intact. × 2.3.

FIG. 2.

Ventral view of ground squirrel with skin and superficial fascia removed. × ½.

FIG. 3.

Same as Fig. 1 with sac contents omitted. Sac routinely severed at level of solid line for weighing. × 2.3.

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[1] Johnson, G. E., Foster, M. A., and Coco, R. M., *Trans. Kans. Acad. Sci.*, 1933, **36**, 250.

[2] Wells, L. J., *Anat. Rec.*, 1935, **62**, 409.

of paired sacs in males with spermatozoa ranged from 0.1212 to 0.5215 gm., yet this weight was slightly less than the average for males lacking spermatozoa in only 2 of 32 cases. The greatest weight of paired sacs in any aspermatic male was 0.2400 gm., and this is significantly less than the average weight for animals with spermatozoa. Data for individual animals indicate clearly that variations in sac weight lag behind variations in testis weight. The testis may be experimentally caused to remain spermatogenetically active within the scrotum during the non-breeding period by subjection of the animal to cold temperature and darkness[3]; such males have large scrotal sacs.

TABLE I.
Weight of Fresh Fibro-muscular Scrotal Sacs of Unoperated Males.
Data expressed as averages for each group.

Age	Month of Autopsy	No. of Animals	Body Wt., gm.	Fresh Wt. of one testis, gm.	Spermatozoa	Weight of 2 scrotal sacs, gm.
Adult	Jan.-May	32	228	.8654	Present	.3338
	June-Dec.	18	208	.1320	Absent	.1338
	Aug.-Dec.*	8	190	.8515	Present	.3044
Young	August	4	137	.0453	Absent	.0438
	October	2	174	.1905	,,	.1440
	January	1	222	.3475	,,	.1717

*Experimental animals exposed to constant temperature of approximately 4° C.

Microscopical studies indicate that weight of the fibro-muscular sac depends on the amount of skeletal cremasteric muscle. Whether variation in the amount of muscle is due to hypertrophy or hyperplasia, or both, has not been determined, but this question is being investigated.

The fibro-muscular sac has been experimentally emptied of its contents by unilateral castration and by production of unilateral cryptorchidism, in prepuberal males. Such animals were operated during September-October and autopsied during February-March when the untreated testis had become large, scrotal and spermatic. The empty sac was invariably small in comparison with the normal contralateral one (Table II). These experiments indicate that the sac does not undergo its maximal development when its contents are experimentally removed.

Does the fibro-muscular sac depend upon testis hormone for its maintenance? This question was experimentally investigated by removing either one testis or both testes from each of 11 males possessing spermatozoa and functional accessory reproductive or-

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[3] Wells, L. J., *Anat. Rec.*, 1935, **64** (Suppl.), 138.

TABLE II.
Effects of Removing the Testis from the Sac in Prepuberal Males.
Data expressed as averages for the group.

No. of Animals	Month of Autopsy	Body Wt., gm.	Wt. of one normal intact testis, gm.	Spermatozoa	Weight of fresh fibro-muscular sac, gm.	
					Empty sac	Normal sac
8*	Feb.-Mar.	245	1.0205	Present	.0869	.2014
5†	Feb.-Mar.	240	.8047	”	.0732	.1493

*Unilaterally castrated.
†Unilateral cryptorchidism experimentally produced.

gans. Testis removal occurred on April 2, and the animals were killed 31 days later on May 3. Average weight of paired sacs of bilateral castrates was only approximately 33% as great as that in normal males with spermatic testes (Tables III, I). Weight of the empty sac of unilateral castrates was invariably less than that of the normal one.

That these results were not elicited by destruction of vascular and nervous elements of the sac during the surgical removal of the testes is suggested by the fact that the empty sac weighed only slightly less than the normal one in unilateral castrates. It seems that other factors were operating. Since the average weight of paired sacs from bilateral castrates was less than the average weight of the empty sac of unilateral castrates, one logically assumes that testis

TABLE III.
Effects of Testis Removal on the Fibro-muscular Scrotal Sac in Adults.

Animal No.	Body Wt. at autopsy gm.	Wt. of one testis removed at operation	Wt. in gm. of intact testis at autopsy	Wt. of seminal vesicles, gm.	Wt. of fresh sac in gm.	
					Empty sac	Normal sac
1388*	210	.9318	.9399	.6013	.1304	.1638
1389*	195	.4918	.5155	.9729	.1151	.1527
1391*	215	.7155	.7418	.7838	.1531	.2214
1392*	205	.6461	.6358	.9828	.1538	.1794
1393*	195	.7445	.6738	1.1884	.1341	.1822
Aver.	204	.7059	.7013	.9058	.1373	.1799
1394†	185	.5437		.0496	.0809‡	
1395†	200	.4482		.0926	.0962‡	
1396†	225	.7340		.0598	.1094‡	
1397†	204	.4473		.0691	.1203‡	
1398†	202	.7015		.0431	.1247‡	
1399†	232	.6903		.0657	.1781‡	
Aver.	208	.5941		.0633	.1182‡	

*Unilaterally castrated.
†Bilaterally castrated.
‡Total weight of both sacs.

hormone is a major factor in sac maintenance. Animal 1399 (Table III) is of special interest, however, because the epididymis was removed from the sac on one side, and was left intact in the other; the empty sac weighed 0.0799 gm. while the other weighed 0.0982 gm. That the weight of the empty sac in unilaterally castrated animals is not due to a subminimal amount of testis hormone is suggested by the fact that seminal vesicles were entirely typical of those found in normal breeding males. It seems probable, or at least possible, that some of the decreased weight of sacs in both unilateral and bilateral castrates may be due to a decreased tonus of the cremasteric fibers, perhaps due to an absence of mechanical stress following testis removal.

It is concluded from these results that the ascent of the testes in adults of this species is not primarily due to scrotal failure, since the scrotum appears to be dependent on the testis. Perhaps the decrease in testis weight is the primary factor causing ascent, but the results of the present study do not prove that it is. The evidence suggests that testis hormone is responsible for maintenance of the sac. Decrease in the output of testis hormone, which has been previously found to closely follow testicular regression in normal males,[3] could account for the decrease in the weight of the sac. But the character of the functional sac is not entirely determined by testis hormone; mechanical stress produced by mere presence of the testis within the sac appears to be a factor in both development and maintenance of the fibro-muscular scrotal sac. Variation in the weight of the sac is caused by fluctuation in the amount of skeletal muscle. Whether this variation is due to hypertrophy or hyperplasia of muscle fibers is being investigated.

9337

Amino Acids (Natural and Synthetic) as Influencing Hemoglobin Production in Anemia.

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We wish to submit evidence that histidine and phenylalanine (both natural and synthetic forms) may under certain conditions exert a definite influence upon the regeneration of red cells and hemoglobin in standardized dogs made anemic by blood withdrawal. We have been accumulating experimental data relating to the influence of various amino acids by mouth upon hemoglobin production and hope to publish a report in the near future.

Many of the dogs used had been under observation for more than 6 to 8 years continuously anemic and carefully standardized with liver, iron and other food factors. In these dogs upon the experimental regimen recently described[1] fluctuation of 10 gm. hemoglobin production per 2-week period may come within physiological limits but fluctuations of 20 gm. hemoglobin or over have significance. It will be noted in Tables I and II that an increase in hemoglobin output above the basal control levels associated with amino acid feeding may amount to 25-50 gm. hemoglobin per 2-week period. The standard salmon bread[1] was fed during all these diet periods and the amino acid added to the first portion of this diet to insure its prompt total consumption. Pure crystalline amino acids were used in these experiments.

TABLE I.
Histidine Dihydrochloride and Hemoglobin Production in Anemia.

				Control Net Hemoglobin Output per 2 wks.		
Dog No.	Amino Acid by mouth	Daily dose fed	Hemoglobin Net Output per 2 wks.	Iron 40 mg. daily-oral	Liver 300 gm. daily-oral	Basal bread ration alone
		gm.	gm.	gm.	gm.	gm.
27-236	l-natural	1	29	57	86	10
23-1	l ”	1	34	46	107	14
27-238	l ”	1	44	59	—	20
30-121	l ”	2	0	46	87	30
29-326	l ”	2	28	46	65	12
27-238	d-optical isomer	1	33	59	—	20

It has been believed by chemists and physiologists that the natural forms of the amino acids are much more active in protein metabolism than the optical isomers or the synthetic dl forms. The evidence relating to hemoglobin production (internal protein synthesis) indicates that in an emergency (anemia) the dog can use all forms of certain amino acids to increase the hemoglobin production.

Histidine (the natural or l form) obviously has an effect when given in one gm. doses per day for a 2-week period. The increase in hemoglobin production due to this amino acid (Table I) approximates ⅓ the increase due to liver feeding in control periods. A single experiment with histidine (the optical isomer or d form) shows a similar effect upon hemoglobin production and other experiments are in progress. The presence of this amino acid in these amounts added to the standard ration enables these standardized dogs to produce much more hemoglobin per week. The mechanism of this reaction is obscure but it may well be argued that this amino acid is of importance in the fabrication of the large globin molecule which makes up the bulk of the hemoglobin molecule. From other experiments with bile fistula dogs it appears that the anemic bile fistula dog can produce readily large amounts of the pyrrol nucleus.[2]

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[1] Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1936, **115**, 651.

TABLE II.

Phenylalanine and Hemoglobin Production in Anemia.

				Control Net Hemoglobin Output per 2 wks.		
Dog No.	Amino Acid by mouth	Daily dose fed	Hemoglobin Net Output per 2 wks.	Iron 40 mg. daily-oral	Liver 300 gm. daily-oral	Basal bread ration alone
		gm.	gm.	gm.	gm.	gm.
29-326	l-natural	1	18	46	82	12
29-326	d-optical isomer	1	27	46	82	12
26-102	dl-synthetic	0.5	12	39	84	14
26-102	’’ ’’	1	25	39	84	10
30-116	’’ ’’	1	51	42	91	14
29-326	’’ ’’	1	36	46	82	12
23-1	’’ ’’	1	8	46	81	16
23-1	’’ ’’	2	11	46	81	16
27-238	’’ ’’	3	29	38	—	24

Table II shows that phenylalanine may give a response when fed as the d or l or dl form. Of the 7 experiments with the dl form we accept 3 as negative (below 20 gm.) and the other 4 as positive (25-51 gm. hemoglobin). It is at least possible that the amino acid may be more or less potent in any given period depending upon the store of amino acids or aggregates at hand in the liver or other body tissues. More experiments with the natural form of phenylalanine are in progress.

Dogs under these conditions show individual variations in their response to food factors or iron. For this reason we give for each dog its individual response to the 40 mg. daily dose of iron and a 300 gm. feeding of liver. On the average the response to this dose of iron is about 50 gm. hemoglobin and for liver about 95 gm. hemoglobin per 2 weeks. A dog which gives a high output of hemoglobin following a standard dose of liver or iron is apt to react above the average to other food factors. We speak of dogs as overactive or subnormal in their response with hemoglobin production.

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[2] Hawkins, W. B., Sribhishaj, K., Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Physiol.*, 1931, **96**, 463.

It may be objected that amino acids added to a diet containing proteins of unknown amino acid make-up give a confused picture of no value. No such objections are made to experiments where iron or copper are added to various diets in anemia due to blood loss or deficient diets. Moreover the internal metabolism of proteins is almost limitless in its complexity and we are merely adding one factor to a host of others in even the simplest type of experiment. To illustrate, an anemic dog during a complete fast with no intake of food factors whatever will produce a considerable amount of new hemoglobin and if given some iron during a fast of 2 weeks will often produce more than 100 gm. of new hemoglobin. This must come from body protein and it has been shown that this new hemoglobin is in part related to the materials which otherwise would appear in the urine as waste products (urea and ammonia fraction).[3] This remarkable conservation of end products and exchange of materials within the body during a fast is an index of what the body can do and indicates the complexity of the internal protein metabolism related to hemoglobin production. The addition of an amino acid to this complex reaction may give a significant response which must be accepted as a fact even if the explanation is unpalatable.

9338 P

Transplantation of Gonads from Lethal to Normal Larvae in *Drosophila melanogaster*.

ERNST HADORN.* (Introduced by B. H. Willier.)

From the Department of Zoology, University of Rochester.

Those lethal genes which allow the individual to develop up to a certain stage before death occurs are of particular interest in analyzing experimentally the problem of gene action during ontogeny.

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[3] Daft, F. S., Robscheit-Robbins, F. S., and Whipple, G. H., *J. Biol. Chem.*, 1933, **103**, 495.

* Fellow of the Rockefeller Foundation. For various kinds of assistance I am deeply indebted to Dr. G. W. Beadle (Harvard), Dr. C. B. Bridges (Pasadena), and Dr. B. H. Willier and Dr. Curt Stern (Rochester).

In such a study we have to deal with the question whether the lethal genes affect unfavorably the development of all organs or whether the cessation of development is the result of their deleterious action on certain organs only, which then would inhibit also the further development of those organs in which the genetic constitution has no primary lethal effect.

In investigating this problem the recessive mutation *"lethal-giant"* (—*lgl*—discovered and located by C. B. Bridges, 1933, Chromosome 2, *locus* 8.2—unpublished) was found to be suitable. The homozygotes show externally a normal development during larval life up to the time of pupation. Unable to pupate normally, they survive as full grown larvae for several days. Usually they are very transparent and bloated. This appearance is the result of an abnormally small fat body and of an accumulation of body fluid. From 4 to 6 days after the time when normal pupation takes place, a few of the lethals die as larvae, but most of them form "pseudopupæ". These have a hardened and darkened cuticle within which no further development occurs.

In the lethal larvae the imaginal discs degenerate completely, while the larval organs show a normal histological structure. From these facts it may be inferred that the lethal gene produces a fatal effect only on the anlagen of the imago, which differentiate later than the larval organs. Of special interest are the gonads which form early in development like the larval organs, but continue their development into the imago in genetically normal individuals. Whether the gonads which are parts of the lethal larva can develop normally may be ascertained only by experimental means. At the third larval instar of such lethals the ovaries are normal in structure and size while the testes show considerable inhibition of growth. The developmental potencies of both ovaries and testes were tested by transplanting them into genetically normal larvae of the same age. The method of Ephrussi and Beadle[1] which was used, allows full development of both ovaries and testes in case they are of normal constitution. The host flies were dissected and the graft examined several days after hatching.

Transplantation of ovaries: From a total of 41 transplants made, 36 ovaries were found. In every case the ovary has developed to a *considerable degree* beyond the stage to which it can go in a lethal larva. The transplants grow and elongate; the egg strings differentiate and their apical ends exhibit contractive movement as in the normal. However the development attained is not complete. The transplants never reach the size of a full grown normal ovary and, although eggs are formed, they degenerate before reaching their full size. It makes no difference whether the ovaries are attached to the host oviducts or are free.

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[1] Ephrussi, B., and Beadle, G. W., *Am. Nat.*, 1936, **70**.

Transplantation of testes: Out of a total of 32 testes transplanted not one developed. In most cases the transplant was not found at all. In a few cases only a small vestigial part of it could be dissected out from the host. These failed to differentiate further.

Conclusions. The distinct but limited development of the transplanted ovaries shows that this organ still has developmental potencies at a time when other organs, such as the imaginal discs, degenerate. This means that *the lethal genetic constitution prevents further development in different organs at different periods of time.* Whether the further development of the ovaries is based on autonomous potencies or whether it is a result of a favorable influence coming from the genetically normal host cannot be determined from these experiments.

The striking difference found between the developmental potencies of transplanted ovaries and testes seems to be correlated with the fact that prior to transplantation, the testes during the third larval instar already show such deleterious changes brought about by the lethal conditions as to prevent their increase in size, whereas the ovaries seem not yet affected.

9339 P

Resuscitation of the Heart from Ventricular Fibrillation with Drugs Combined with Electric Shock.*

FREDERICK R. MAUTZ. (Introduced by C. S. Beck.)

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Hoffa and Ludwig[1] demonstrated that electrical stimulation of the mammalian heart led to ventricular fibrillation and death. Prevost and Battelli[2] studied the effect of electric currents on the heart and reported that a brief countershock of sufficient strength applied directly to the heart through an electrode placed on the ventricles would produce a brief period of complete cardiac standstill from which coördinated ventricular beats might be resumed. Cardiac massage was found necessary preceding the countershock when the ventricular fibrillation had existed longer than 15 seconds. Hooker, Kouwenhoven and Langworthy[3] found that currents of 0.4 amperes and less applied directly through the heart cause ventricular fibrillation, while currents of 0.8 amperes and over will stop the fibrillation. They advised the central carotid injection of calcium chloride and adrenaline after countershock as an aid in restoring a vigorous beat. Wiggers[4] emphasized the importance of cardiac massage preceding the electric shock to improve the nutrition of the myocardium and he reported success in reviving 85% of fibrillating dog hearts.

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* Aided by a grant from the Josiah Macy, Jr. Foundation.

[1] Hoffa, M., and Ludwig, C., *Z. f. rat. Med.*, 1850, **9**, 107.

[2] Prevost, J.-L., and Battelli, F., *Comptes Rendus des Séances de L'Académie des Sciences*, 1899, **129**, 1267.

The method of cardiac massage and electric shock with a current of approximately 1.5 amperes applied through the heart, with electrodes 3.5 by 9 cm. placed on each side of the heart, was used in the surgical laboratory in cases of ventricular fibrillation developing in the dog's heart during experimental cardiac surgery. Bare metal electrodes were used on the heart; they do not cause local burning if there is a large area of contact with the heart. It is preferable to make the electrodes of a metal that will not corrode. Success was obtained in about half the cases, but there were many failures, the cause of which was not apparent. After it was determined that procaine hydrochloride is a myocardial depressant[5] and readily absorbed from the epicardium, this drug was applied over the surface of the heart in doses up to 20 mg. per kilo of body weight in the hearts difficult to revive, and it was found that a higher percentage could be revived. It was later found that by giving doses up to 10 mg. per kilo intravenously practically every case of ventricular fibrillation could be restored to a coördinated beat with the electric shock. No central nervous toxic effects were observed.

In the heart difficult to revive from ventricular fibrillation the following considerations are important: (1) Artificial pulmonary ventilation must be adequate to saturate the blood with oxygen. (2) Cardiac massage must be effective in producing sufficient circulation to nourish the brain, prevent intravascular clotting, maintain vigorous fibrillations in the myocardium, and prevent or overcome extreme cardiac dilatation. (3) If countershock is now unsuccessful, intravenous procaine will produce a coarser type of fibrillation which can more readily be restored to a coördinated beat with an electric shock. (4) In extreme dilatation which may follow a toxic dose of procaine, dilute solutions of calcium chloride and adrenaline in small doses are antagonistic and aid in producing the necessary condition for restoration of a coördinated beat. (5) When a feeble beat has been restored, gentle massage and, if this is ineffective, cautious administration of calcium chloride and adrenaline[6] aid in restoring a vigorous heart beat. When procaine has been given previous to electric shock, there is little tendency to return to fibrillation unless excess calcium chloride is introduced. The heart must be carefully observed, for a period of about 20 minutes before the chest is closed, for acute dilatation during the recovery phase.

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[3] Hooker, D. R., Kouwenhoven, W. B., and Langworthy, O. R., *Am. J. Physiol.*, 1933, **103**, 444.

[4] Wiggers, C. J., *Proc. Am. Physiol. Soc.*, 1936, p. 161.

[5] Mautz, F. R., *J. Thoracic Surg.*, 1936, **5**, 612.

In summary, a proper state in the fibrillating ventricular myocardium can be obtained by the careful use of myocardial depressant drugs and their antagonists, which makes it possible almost routinely to revive the heart from ventricular fibrillation. The chances of permanent recovery depend upon the magnitude of the operative procedure. In 25 consecutive cases of ventricular fibrillation in dogs the author has been able to restore a normal heart beat by using the foregoing technique. Many of the hearts were severely damaged by contusions, stab-wounds, etc. As long as 30 minutes have elapsed before revival with complete postoperative recovery, and no signs of cerebral injury. Even though difficult to develop, this technique is feasible and should be available for use in the operating room in cases of ventricular fibrillation.

9340 P

Quantitative Studies on Nerve Regeneration in Amphibia. I. Factors Controlling Nerve Regeneration in Adult Limbs.

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Nerve regeneration is the result of a growth process starting from the cut ends of those parts of the severed neurones left in connection with their cell bodies. Extensive bifurcation of the outgrowing sprouts is supposed to provide for overabundance of peripheral terminations. To what extent the excess of innervation is kept within bounds, and by what factors, has not yet been determined. Observations showing that the number of regenerated nerve fibers in supernumerary transplanted limbs is of the order of that of a normal limb, irrespective of the size of the nerve source, (Weiss[1]) suggested the existence of peripheral influences controlling the amount of fiber regeneration.

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[6] Hooker, D. R., *Am. J. Physiol.*, 1930, **92**, 639.

Following this lead, a series of experiments was undertaken on adult specimens of the Japanese newt, *Triturus pyrrhogaster* (Boie). Operations were performed on the brachial plexus (spinal nerves 3, 4 and 5) of one side, the opposite side serving as control. After the operation, 120 days were allowed for nerve regeneration, whereupon experimental and control limbs were fixed and treated by Bielschowsky's silver method. Nerve counts were made in cross sections at 3 representative levels (H, *proximal humerus;* E, elbow; W, proximal wrist), and the percentage difference between the number of regenerated fibers in the operated limb and the number of original fibers in the control limb was calculated.

Experiment I. (7 cases.) Transection of all brachial nerves at the shoulder level. A fiber deficit was found in the regenerated nerves, the average percentage difference amounting to, at H: 15.4%; at E: 27.2%; at W: 45.6%. This deficit seems to parallel a certain atrophy of the denervated and re-innervated limbs, the average reduction of their cross-sectional area being 21.5%. The number of nerve fibers entering the limbs (at level H) is, therefore, grossly speaking, of the same order before and after nerve regeneration.

Experiment II. (5 cases.) After transection of the brachial nerve plexus, the proximal stump of the fourth spinal nerve which is the strongest of the plexus, was inserted into shoulder muscles so as to prevent it from contributing fibers to the free limb. By this means the source of fibers available for the latter was restricted to the third and fifth nerves, which meant a reduction to about one-half of the source available in experiment I. After 120 days, the average percentage differences were found to be, at H: 20.2%; at E: 50.8%; at W: 52.2%. These figures show that the conspicuous reduction of the amount of fiber stumps available for regeneration has failed to cause a corresponding, or even an appreciable, decrease in the number of fiber branches entering the limb (at level H; compare with Exp. I).

Experiment III. (5 cases.) After transection of the brachial nerve plexus the distal fragments of the severed nerves were extirpated as far distally as possible, eliminating thus a great deal of the pathways (Bungner's cords) preferentially followed by regenerating fibers. Here, the deficit was considerably greater than in experiment I, namely, at H: 42.2%; at E: 67.2%; at W: 67.5%.

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[1] Weiss, Paul, *J. Comp. Neurol.*, 1937, **66**, 481.

Experiment IV. (6 cases.) Combining the operations of Exp. II and Exp. III. The differences were, at H: 39.4%; at E: 64.7%; at W: 68.8%. Comparing these figures with those of Exp. III, one notices again that the reduction of the proximal nerve source has failed to entail a corresponding reduction in the amount of fibers found within the limb. The absence of the peripheral sheaths, on the other hand, has caused in every case a material reduction in the peripheral supply (compare Exps. II and IV with I and III).

The data obtained seem to indicate that, (1) the branching of regenerating fibers is extensive enough to permit practically full reinnervation of a limb even from an undersized nerve source; (2) the degree to which a limb is repleted with regenerated fiber branches is controlled by factors residing within the limb and tending to limit the actual supply to approximately the normal amount; (3) the degenerating peripheral nerves may represent one major factor exerting numerical control over the admission of new fiber branches; but even in their absence the limb tissues seem to continue to exhibit a certain degree of control.

9341 P

Quantitative Studies on Nerve Regeneration in Amphibia. II. Innervation of Regenerated Limbs.

PAUL WEISS AND RAYMOND LITWILLER.

From the Department of Zoology, The University of Chicago.

When the developed limb of a urodele amphibian is amputated, masses of mesenchym accumulate at the wound and by subsequent growth and differentiation produce a new limb. Concomitantly, nerve fibers sprouting from the severed old nerve trunks invade the regenerating limb and provide its nerve supply. This process, studied in a preliminary manner by Weiss and Walker,[1] was given a more thorough examination in the present study.

Adult specimens of the Japanese newt, *Triturus pyrrhogaster* (Boie) and Mexican Axolotls were used, the latter in 2 different size (age) groups (75-85 mm. and 120-150 mm. in length). Fore limbs were amputated at the body wall and allowed to regenerate. After varying periods of time, the regenerated limbs were preserved and sectioned. In the newt, Bielschowsky's silver stain for axons proved successful, while the data concerning the Axolotl are based mainly on myelin stains (Weigert-Pal). Tests in which 2 opposite limbs of the same animals were treated by the 2 different methods, staining on one side all fibers, on the other side the medullated fibers only, revealed that in regenerated fore limbs, 75-80% of the fibers are medullated. Every 20th cross section (cut at 10 micra) was measured, and the amount of nerve fibers contained therein was counted.

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[1] Weiss, P., and Walker, R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 810.

The number of nerve fibers present in the old limb base proximal to the level of amputation was rather constant: cca. 900 and 1200 fibers in the smaller and larger Axolotls, respectively. Distally to this level, in the regenerate, the number declines gradually in such regular manner that a definite relation between nerve supply and mass of innervated tissue suggested itself. The relation was revealed by the graphs in which the data were plotted, to be a remarkably simple one: If a regenerate is divided up into a series of slices of 200 micra each; if v_s is the mass of one of these slices; and if the number of nerve fibers terminating in this slice (determined as the difference between the nerve numbers counted in the 2 levels bounding the slice) is n_s; the ratio

$$k = \frac{n_s}{v_s}$$

was found to be nearly constant for all slices of a regenerate of a given age. In other words, the density (k) of nerve endings as well as, reciprocally, the mass of tissue innervated by a single fiber (1/k) are constants for a given limb. In a specific example, every slice of a regenerate of 50 days (Axolotl, 130 mm.) was calculated to contain 47 nerve terminations per one cmm. of volume. The factor k was found to decrease consistently with increasing length of regeneration. Furthermore, it is smaller in older than in younger animals.

The reported facts indicate that the tissues of a regenerating limb exert a definite control over the admission of new nerve fibers, establishing a "saturation" point for the number of possible terminations in a given peripheral volume. The fact that the saturation coefficient (k) decreases as regeneration proceeds, indicates that nerve fibers effect connections with the peripheral tissues at a very early stage of regeneration, and that the relations thus established are not essentially disturbed by the continued growth of the innervated tissue, largely muscle.

9342

On the Nature of the First Forelimb Movements of Mammalian Embryos.

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From the Anatomical Laboratory, Northwestern University Medical School.

Somatic movements occurring in response to faradic stimulation were studied in cat embryos delivered by experimental Caesarian section. The cats were decerebrated by ligating carotid and basilar arteries, after which one to 3 hours were allowed for recovery from ether anesthesia. Embryos were delivered as promptly as possible and examined while their placental circulation was intact and before uterine contractions had caused placental separation. All experimentation was carried out in a bath of physiologic saline solution maintained at the temperature of the cat's body. The combined diameter of the nichrome-wire stimulating electrodes was less than one millimeter and they were insulated except at their tips. Strength of current was not measured but could be borne upon the tongue. At the conclusion of physiologic studies, the embryos were prepared for histologic examination by the pyridine-silver technique.

It was seldom possible to obtain reflex movements in embryos less than 14 mm. long. But when the electrodes were placed directly over developing muscle masses of the shoulder region contractions were elicited with perfect regularity. Motor and sensory spinal nerves are well formed at this stage; free nerve endings appear beneath the epithelium of the forelimb and a few primitive endings are present upon the myoblasts of the shoulder muscles. Within the spinal cord, motor and associational mechanisms are well represented but morphologic reflex arcs are incomplete because the sensory dorsal funiculus collaterals are just forming and have made no connections with other neurons.

In embryos of about 23 days' insemination age (14 mm.), the sensory nerves have grown down the dorsal and volar aspects of the forelimb to within half a millimeter of its most distal point. There they end in free nerve endings immediately beneath the epithelium. There is little muscle formation distal to the shoulder but in the shoulder muscles many free and grapelike endings were observed upon the myoblasts. In the spinal cord, unisegmental structural reflex arcs have been completed by the growth of sensory collaterals into the groups of associational neurons of the dorsal gray matter and

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* Aided by a grant from the American Academy of Arts and Sciences.

even to the motor neurons which supply the forelimbs. When, in these embryos, the points upon the hand reached by the new sensory nerve fibers were stimulated with the wire-electrodes a quick outward movement at the shoulder sometimes followed. This movement was not accompanied by any others, although a local extension of the head separately could sometimes be obtained by stimulating the nose region in a similar manner. Stimulation of points upon the body not yet reached by sensory nerves or at levels whose spinal cord segments contained less mature structural reflex arcs never resulted in movements of this type.

The responses in question were distinctly different from those obtained by direct stimulation of muscle masses. They exhibited fatigue to a degree not seen with direct stimulation, a just perceptible latent period following the stimulus was often seen, and they were definitely stereotyped and invariable reactions. Furthermore and of primary importance, they could be obtained only in the first few minutes of the experiment before the uterus had contracted upon the placenta. Contractions due to direct muscle stimulation occurred in dead embryos; but even in mildly depressed living specimens or in those injured slightly during delivery no movements occurred in response to localized stimulation of receptor endings. If spread of current were the cause, all should have shown the phenomenon. There can be no doubt that the movement in question is a true reflex of the simplest type involving afferent conductor, center, efferent conductor and effector.

By 26 days (18-20 mm.), a variety of reflex responses to faradic stimulation could be obtained. Stimulation of the tip of one paw often produced local abduction of the ipsilateral forelimb at the shoulder with or without flexion at the elbow, it sometimes produced contralateral forelimb reflexes, and frequently movements of the head. Stimulation of the dorsal aspect of one hand was followed by an ipsilateral forelimb reflex and ipsilateral head flexion; stimulation of the volar aspect caused contralateral head flexion. Reflexes always involved muscles at the same level or at levels rostral to that stimulated, but not caudally. These physiologic observations are in harmony with what is known concerning the histologic structure of embryos at this time. It has been shown[1] that the early fiber tracts of the embryonic spinal cord pass rostrad, and only later is there a rostral to caudal integration of structural reflex patterns. It has further been shown that the first structural reflex arcs are unisegmental, involving sensory collaterals from the lateral part of the dorsal funiculus which terminate at approximately the level at which their dorsal roots enter the cord. In the 18 mm. embryos, the ascending branches of the dorsal root fibers give off collaterals farther rostrad which make contact with commissural neurons whose discharge would excite motor cells supplying the neck muscles.

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[1] Windle, W. F., *J. Comp. Neurol.*, 1933, **58**, 643; 1934, **59**, 487.

Barcroft and Barron[2] have reported some experiments in sheep fetuses in which they dissected the median nerve and the lateral popliteal nerve, severed them, and stimulated central and peripheral ends. They found that no movements followed stimulation of the central end of the median nerve in sheep younger than 44 days old and none followed similar stimulation of the lateral popliteal before 47 days. They concluded, therefore, that the primary afferent neurons distributed to the limbs have not made functional connections with the anterior horn cells prior to these times and that any movements seen earlier are not true reflexes. It would seem that negative evidence obtained after mutilating operations upon such delicate forms as sheep embryos younger than 44 days old cannot serve as sound basis for the conclusions they have drawn. The tissues of sheep embryos 38 days (31-32 mm.) to 45 days old (57 mm.) are very susceptible to injury. Even if it were possible to keep the uterus from contracting upon the placental cotyledons (and setting up a state of partial anoxemia) for the time necessary to complete a dissection of the nerves in question, and even if such dissections could be made without loss of a significant percentage of the small total blood volume of the fetuses, the trauma involved in the procedures would certainly be such as to render the nervous system of the specimens no longer irritable to faradic stimulation.

It may be concluded from the experiments in cat embryos that early forelimb movements in response to faradic stimulation of the epithelium over newly formed sensory nerve endings of the hand are true spinal type reflexes. These reflexes are not part of a total mass reaction pattern when they first appear. But with progressive development of central reflex mechanisms more complex and integrated reflexes become elicitable.

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[2] Barcroft, J., and Barron, D. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 86.

9343

Significance of Peripheral Receptors for Excitability of the Central Nervous System.

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Galkin[1] published extensive investigations in which he tried to show that the elimination of the olfactory receptors in dogs produced a permanent state of somnolence and, finally, death in the animal. The removal of the eyes and the destruction of both cochleæ did not have any such effects. He assumed that the elimination of those sense organs which are most important for the normal behavior of the animal are responsible for the lethargic state observed in his experiments. These observations seem to be in contrast with experiences of Goltz,[2] Rothmann,[3] Dusser de Barenne,[4] and others, who did not observe such a state in dogs and cats whose hemispheres had been removed and in which the olfactory nerves had also been severed. Pavlov[5] stated that a dog deprived of the anterior half of its hemispheres showed, at least after some months, a nearly normal locomotion, and even states of excitement during periods of hunger. He did not mention any lethargic conditions in spite of the severance of the olfactory nerves. In view of the principal importance of the question as to the significance of the biologically most important sensory nerves for the general excitability of the central nervous system and the behavior of the animal it seemed advisable to repeat Galkin's experiments.

We have operated 4 dogs in which the olfactory nerves were severed and we employed the same method which Galkin described. Careful histological investigations were carried out which were omitted by Galkin. The result of these histological studies is that in 2 of our dogs the olfactory nerves were completely severed, whereas in 2 others some fibers were still intact. The dogs were observed over a period of 1-4 months. Except for one case in which a somewhat somnolent state of the dog was observed for several days, a state which was accompanied by a nasal discharge but with no fever, the dogs appeared to be completely normal in their behavior and excitability for several weeks or months before they were sacrificed. These observations do not lend any support whatever to the statement of Galkin, and make it not improbable that his results are due to some chronic infections which may have been overlooked, since a histological study of the brain was omitted. We add here the microscopic findings of the 2 dogs which showed complete severance of the olfactory nerves.

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[1] Galkin, W. S., *Z. f. exp. Med.*, 1933, **88**, 316.

[2] Goltz, F., *Pflüger's Arch.*, 1892, **51**, 570.

[3] Rothmann, M., *Neurolog. Zentralblatt*, 1912, **31**, 867.

[4] Dusser de Barenne, J. G., *Arch. Neerland.*, 1919, **4**, 31.

[5] Pavlov, I. P., *Conditioned Reflexes*, Oxford Univ. Press, 1927, p. 365.

Dog A. Both frontal lobes and olfactory bulbs show areas of organized anemic softening, which had produced a severe destruction of the olfactory bulbs. In the latter the characteristic histologic pattern has been replaced by a diffuse infiltration with glia nuclei and overgrowth of collagenous connective tissue fibers. The pia-arachnoid covering of the frontal lobes is thickened, but no inflammatory reaction is present.

Dog D. There is a severe destruction of both olfactory bulbs. The inner cavities are markedly enlarged and only a thin wall of tissue remains. The main part of the bulbs is destroyed by anemic softening and large amounts of compound granular corpuscles are seen. The olfactory nerves as they pass through the lamina scribrosa are degenerated. They show a considerable increase in elongated nuclei and a mild proliferation of endoneurial connective tissue. No inflammatory reactions are seen.

The experiments were extended to rabbits and were carried out on 6 animals, 2 adult and 4 about one-month-old rabbits of the same litter. In these animals the olfactory bulbs were completely removed. The animals were observed in the laboratory for about one year and did not show any abnormal behavior. The growth curves of the young rabbits were very similar to that of other rabbits of approximately the same age. These observations seem to agree with the work of Swann,[6] who studied effects of the destruction of the olfactory tracts and various parts of the archipallium on the retention of olfactory habits in rats. He finds that the destruction of the primary olfactory tracts abolished olfactory habits but failed to observe any other general disturbances.

Conclusion and Summary. The sectioning of the olfactory nerves in dogs and the removal of the olfactory bulbs in rabbits do not cause any general symptoms such as lethargy, as has been claimed by Galkin. The general excitability of the central nervous system remains unaltered after the elimination of olfactory perception in odoriferous animals just as well as the removal of sight and audition does not influence the central nervous system as a whole in the human. The removal of the olfactory bulbs in young rabbits does not interfere with normal growth.

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[6] Swann, H. G., *J. Comp. Neurol.*, 1934, **59**, 175.

9344 P

Nature of Diet in Its Relationship to Control of Dental Caries.

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(Introduced by P. C. Jeans.)

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Previously it has been reported that dental caries may be arrested within 8 to 10 weeks through the regular ingestion of a diet high in protective foods, including a teaspoonful of cod liver oil daily.[1] This report correlates the response of the teeth with the level of intake of certain dietary constituents.

Five orphanage children between the ages of 3 and 6 years were observed in the metabolism ward for 7 months under varied intakes of vitamin D; concomitant dental examinations and balance studies were made at frequent intervals. Two had active caries of noteworthy extent and activity; one had 20, the other 6 cavities. A third child had one small proximal cavity. The remaining 2 children were free from demonstrable tooth decay. Activity of caries was determined on the basis of the permeability of the exposed dentin to the exploring tine; if at all permeable, the decay was considered active.

Throughout the studies the food intake was quantitatively controlled. During the fall months especially the children received considerable sunshine out of doors. Several dietary regimens were used progressively, each for a period of from 5 to 8 weeks.

Diet No. 1 was an approximation of that employed at the orphanage. It provided 800 gm. whole milk daily, with fair amounts of vegetables, fruits, meat and butter. The second diet was that employed in the Children's Hospital; it provided 1000 gm. of milk, and was higher in vegetables and fruits than the orphanage allowance. The third diet was similar to the second, except that 450 cc. of irradiated evaporated milk, suitably diluted (155 units of vitamin D), was substituted for the fresh whole milk. In the fourth period the second diet was supplemented with a teaspoonful of cod liver oil daily. The latter had an assayed content of 175 units of vitamin D per gram, which provided a daily intake of approximately 600 units.

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[1] Boyd, J. D., Drain, C. L., and Nelson, M. V., *Am. J. Dis. Child.*, 1929, **38**, 721.

Appreciable lessening of activity of caries was noted during the first few weeks of study, during the fall months. Subsequently for many weeks the status was virtually stationary, with minimal but definite activity, even after the children had received a diet of high protective value aside from its vitamin D content for 5 months. When this diet had been supplemented by cod liver oil for 9 weeks, the caries was finally adjudged arrested, and this was confirmed by subsequent examination. The 2 control subjects remained free from caries.

Another boy of 11 years was being observed simultaneously regarding the effects of vitamin A intake on dark adaptation. He had 3 cavities of moderate extent and of mild activity when first seen in mid-September. From that time until January he received a diet with a reduced content of vitamin A, with elimination of all cream, eggs, organ meats and carotene-containing fruits and vegetables. It supplied 1100 gm. skimmed milk daily, and designedly met basic requirements of all nutritional factors other than vitamins A and D, yet it was definitely poorer in protective foods than the routine hospital diet. The boy played out of doors in moderation. In order to reduce still further the intake of vitamin A, in January the skimmed milk allowance was reduced to 750 gm. and other minor alterations were made. On February 3rd was instituted the daily administration of 10 drops of viosterol (2400 units), 4 teaspoonfuls of 1% iron ammonium citrate, 250 units of vitamin B and 50 units of vitamin G. This final regimen was continued throughout the remainder of the period of observation. A new dental cavity was noted on an occlusal surface a month after the study was initiated. Possibly slight improvement occurred during the first 9 weeks, but 19 weeks after the first observation there had been slight increase in extent of caries, with minimal activity. Complete arrest did not obtain until early April, after 9 weeks of viosterol administration. A control subject of the same age but with no caries developed no demonstrable caries under the same regimen.

Significance is attached to the time required to establish arrest of caries in these subjects. The hospital diet seems adequate aside from its content of vitamin D; yet since caries was not arrested until the diet had been supplemented for 9 weeks with considerable amounts of that vitamin, one may conclude that the level of vitamin D was the determining factor. It also seems significant that a stage of near-arrest may be attained with moderate control of the diet, whereas more stringent measures are needed to complete and maintain the process of arrest.

9345

Gonadotropic Hormones of the Hypophysis of the Turkey.*

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Relatively little work has been done in the past on the hypophysis of birds. Benoit[1] obtained evidence for the presence of gonadotropic hormones in hypophyses of sexually active ducks but not in those of inactive ones. Similarly Riddle and Schooley[2] found no effects from implanted hypophyses of pigeons less than 1.8 months old, while those of mature pigeons gave definite indication of the presence of some gonad stimulating principle. Since birds develop no true corpora lutea and differ also otherwise in their physiology of reproduction markedly from mammals, it seemed desirable to make a more complete, qualitative and quantitative analysis of their gonadotropic hormones.

Through the courtesy of Mr. A. C. Gingerich, we were permitted to collect about 6000 hypophyses at the packing plant of the Maplecrest Turkey Farms at Wellman, Iowa. The glands were taken from birds about 6 months old, the hens weighing around 10 and the gobblers around 18 pounds. The fresh glands weigh about 13 mg. and 25 mg. respectively. However, the male glands lose more weight in the process of acetone-drying. One day's collection yielded 6.978 gm. dry powder from 1655 female glands and 9.900 gm. from 2047 male glands.

The dry powder taken up in distilled water was assayed in 4 different tests. Table I gives the results obtained from injections into immature 21 day old rats. The largest dose (100 mg.) produces large ovaries which usually contain about as many corpora lutea as mature follicles. (However, in several cases the corpora were more numerous while in one case they were absent.) In the second group (50 mg.) the increase in ovarian weight is almost entirely due to the presence of many stimulated and mature follicles. A few incomplete corpora lutea are found in 3 cases. The third group (25 mg.) has slightly increased ovaries that contain only few mature or distinctly enlarged follicles. However, sections through the vagina prove that enough estrogenic hormones were released to produce

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* Aided by grants from the National Research Council, Committee for Research in Problems of Sex.

[1] Benoit, J., *C. Rend. Soc. Biol. Strasbourg*, 1935, **118**, 672.

[2] Riddle, O., and Schooley, J. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1610.

full cornification in the vaginal epithelia. The lowest dose (10 mg.) produces no visible morphological change in the ovary though it stimulates the endocrine apparatus, as can be seen from the development of the vaginal epithelium. The vaginal stage was in every

TABLE I.

Assay of acetone-dried turkey hypophyses on 21-day rats. The total amount given in the first column was administered in 6 injections during 3 days. The recipients were killed 24 hours after the last injection (except the animals showing vaginal stage 4, which were killed one day later). F, mature follicles; f, enlarged immature follicles; L, corpora lutea; l, luteinizing follicles.

Hypophysis injected	mg.	Wt. of fresh ovaries, mg.		Aver.	Ovarian histology	Vaginal stage
Male	100	19.3	25.1	37.3	FL	3
"	"	30.9			F–	3
Female	"	28.5	33.1		FL	3
"	"	37.7			FL	3
Mixed	"	37.4	48.2		FL	3
"	"	36.1			Fl	3
"	"	71.2			FL	4
Male	50	28.8	27.7	23.6	F	3
"	"	27.6			Fl	3
"	"	32.9			Fl	3
"	"	21.8			F	3
Female	"	13.5	18.1		F	3
"	"	25.0			F	3
"	"	15.5			F	3
"	"	18.6			F	3
Mixed	"	24.6	26.3		Fl	4
"	"	28.0			F	3
Male	25	14.2	14.8	17.2	f	3
"	"	14.8			f	3
"	"	15.4			f	3
Female	"	15.0	14.8		f	3
"	"	13.5			f	3
"	"	16.1			f	3
Mixed	"	20.4	21.9		f	3
"	"	31.1			f	3
"	"	14.2			f	3-4
Male	10	12.5	11.5	12.1	–	1-2
"	"	10.6			–	1-2
Female	"	14.3	12.7		–	1-2
"	"	11.2			–	1-2
Mixed	"	10.5	12.2		–	1-2
"	"	13.0			–	1-2
"	"	13.0			–	1
Controls		12.4		13.3	–	0
"		14.9			–	0
"		12.9			–	0
"		15.7			–	0
"		13.1			–	0
"		11.8			–	0
"		13.9			–	0
"		12.2			–	0

case ascertained by cross sections. The vagina remained closed even in rats that had received the highest dose. Ovulation was never observed. All corpora lutea that were investigated contained eggs, often fragmented in 2 or more pieces. All 4 dosages cause also enlargement of the uteri. At the lowest dose it is already quite definite; at 25 and 50 mg. the uteri are often widely distended while the highest dose produces greatly thickened uterine walls.

TABLE II.

Assay of acetone-dried turkey hypophysis on 28-day rats. The total amount given in the first column was administered in 6 injections during 3 days. The rats were killed 24 hours after the last injection.

Hypophysis injected mg.		Wt. of fresh ovaries, mg.	Aver.	Ovarian histology	Vaginal stage
Mixed	50	58.0	60.9	FL	4
,,	,,	57.9		FL	4
,,	,,	66.8		FL	4

TABLE III.

Assay of acetone-dried turkey hypophysis on hypophysectomized rats operated 2 weeks prior to beginning of injections which were given over 5 days in 10 equal amounts.

Hypophysis injected, mg.		Wt. of fresh ovaries, mg.		Ovarian histology	Vaginal stage	Wt. in gm. at hypophysectomy	Wt. in gm. at autopsy
Male	100	39.8	31.2	FL	3	101.0	96.3
,,	,,	22.6		Fl	3	100.5	102.0

In a second test the same powder was assayed on 28-day-old rats. While the ovaries of controls have not enlarged during the fourth week, they have become much more responsive; injections of 50 mg. give nearly 3 times as heavy ovaries as in the 21-day rat. The vaginæ open and show stage 4 at the end of the fourth day. Some follicles ovulate, though most of them luteinize while they still enclose the egg.

It is obviously not easy to say just what amount of powder should be considered as having the potency of one rat unit. Since it is customary to use as a test animal the 21-day-old rat in which a distinctly recognizable reaction in ovary, uterus and vagina is produced by 25 mg. (Table I), we may say that this dosage contains one (total) rat unit. It represents the contents of about 6 turkey pituitaries. In view of the work of Benoit and Riddle, one has to consider the possibility that the hypophyses of our birds, which were killed at the very beginning of their first reproductive season, were not yet at the peak of productivity.

The third test was added to make sure that the luteinizing principle was contained in the hypophyseal powder. Two hypophysectomized rats were injected with 50 mg. of turkey pituitary powder in 2 daily injections extending over 5 days. Hypophysectomy had preceded the injections by 2 weeks. Throughout this period the animals remained in dioestrus. On the fourth day of injections, the vaginal smear changed to 3 and remained so until the sixth day, when the animals were killed. At this time the ovaries contained numerous mature follicles, some of them ovulated, and all showed stages of progressive luteinization. The uteri are greatly enlarged. This test removes any possible doubt concerning the actual presence of both the follicle-stimulating and the luteinizing principle in the bird hypophyses. It is of no small interest to note that the gonadotropic hormones are of nearly the same nature in birds and in mammals though the reactions which they control are quite different in the 2 taxonomic classes.

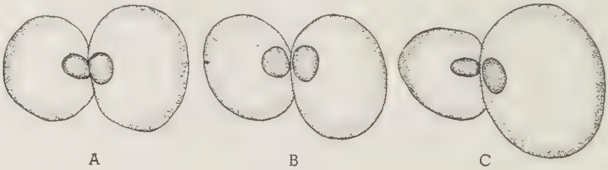

FIG. 1.

Camera lucida drawings of testes of 3 sparrows stimulated by injection of acetone dried, powdered turkey hypophyses, and (inside outlines) of 3 control sparrows which were injected with serum of a pregnant woman, without effect; × 2. Total amounts injected: *A*, 85 mg. turkey hypophysis (15 days), resp. 4 cc. serum p. w. (16 days). *B*, 90 mg. turkey hypophysis (15 days), resp. 4 cc. serum p. w. (16 days). *C*, 130 mg. turkey hypophysis (12 days), resp. 8 cc. serum p. w. (16 days).

In a fourth test the effect of the turkey hypophyses on adult sparrows in the state of sexual quiescence was studied. This condition is characterized in the male by small and inactive testes, thin and only slightly convoluted vasa deferentia and light brown bills, in the female by smallness of the ovaries and oviducts.[3] Six sparrows, 3 males and 3 females, were injected with various dosages of powdered hypophyses over periods of 11 to 15 days. The total amounts (85 to 130 mg.) are equivalent to 3.5-5 rat units. The extensive effects on gonads and secondary sex characters are partly represented in Figs. 1 and 2. The left testes of the males are enlarged at least 60 to 120 times. Spermatogenesis is in full progress; the seminal tubules contain masses of mature spermatozoa. The vasa deferentia are slightly convoluted and enlarged. The bills have turned

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[3] Witschi, E., *Wilson Bull.*, 1935, **47**, 177.

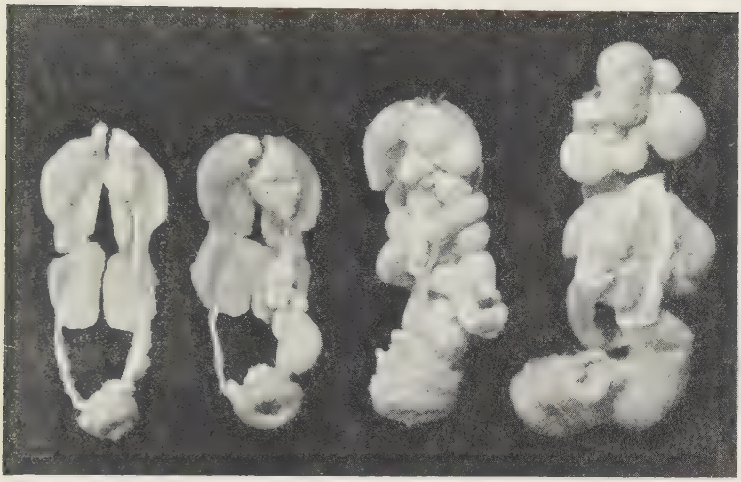

FIG. 2.

Urogenital apparatus of 4 female sparrows, phot. × 1.5. The first one illustrates the quiescent condition, ovary and oviduct covering a minor part of the left kidney; it can serve as control, since the injection of 8 cc. serum p. w. (16 days) has not caused any change. The second and the third received 90 mg. of turkey hypophysis (in 15 days); ovaries and oviducts greatly enlarged. The fourth one received 110 mg. of turkey hypophysis (in 11 days); ovary and oviduct, enlarged to full breeding size, cover the kidneys entirely.

dark blue (stage 2, Witschi and Woods[4]). The corresponding changes in ovaries and oviducts of the females appear clearly in Fig. 2. Control animals were injected with 4-8 cc. of serum of a pregnant woman. This serum contained about 100 rat units of gonadotropic principle per cc. It had no stimulating influence on either gonads or secondary sex characters (Figs. 1 and 2).

From our experiments we can conclude that powdered turkey hypophysis is in quality very similar to corresponding products from cattle, sheep or rats. If equal weights of these powders are compared, the turkey hypophyses appear more potent than bull but less potent than sheep or rat hypophyses. Male and female hypophyses of the turkey show no marked sexual differences in our tests.

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[4] Witschi, E., and Woods, R. P., *J. Exp. Zool.*, 1936, **73**, 445.

9346 P

Effect of Indol on Hematopoiesis in Dogs Fed Deficient Diets.

C. P. Rhoads.

From the Hospital of the Rockefeller Institute for Medical Research, New York City.

Previous communications[1] from this laboratory have presented evidence that anemia can be caused in dogs by administering amidopyrine together with a black-tongue-producing diet. Neither amidopyrine alone nor the diet alone caused anemia under the experimental conditions observed. The anemia which resulted from the combination of drug and diet could be cured by administering a yeast preparation which is preventive of the symptoms of black tongue. The uniform effectiveness of amidopyrine under these circumstances suggested that some endogenous aromatic compound might be similarly effective. Indol was selected for trial because it was available commercially and because certain published experimental evidence was at hand which bore on its mode of detoxification (Houssay[2]).

Dogs were kept under standard conditions and were fed the black-tongue diet which has been described previously.[3] The diet was known to cause symptoms regularly in from 5-12 weeks. Anemia occasionally occurred due to the diet feeding alone, but was of such an incidence and degree that it could be ruled out as a complicating factor in the experiment.

Experiment I. (Fig. 1.) The black-tongue diet was fed without supplement until the first manifestations of stomatitis appeared. Then one gram of crystalline indol was fed daily in a capsule. A very severe drop in blood levels resulted with striking suddenness. Erythrocyte levels of one million cells or fewer per mm.^{3} were encountered frequently and fatalities from acute anemia were not uncommon. If the animal survived the immediate drop in blood levels, there was a distinct tendency toward recovery, as shown by a rise in blood levels in spite of the fact that the indol administration was continued. Control studies in which the same amount of indol was administered to dogs fed a normal diet resulted in no persistent anemia of significant degree.

Experiment II. (Fig. 2.) Prolonged experiments were next made in order to establish beyond question the effect of diet on

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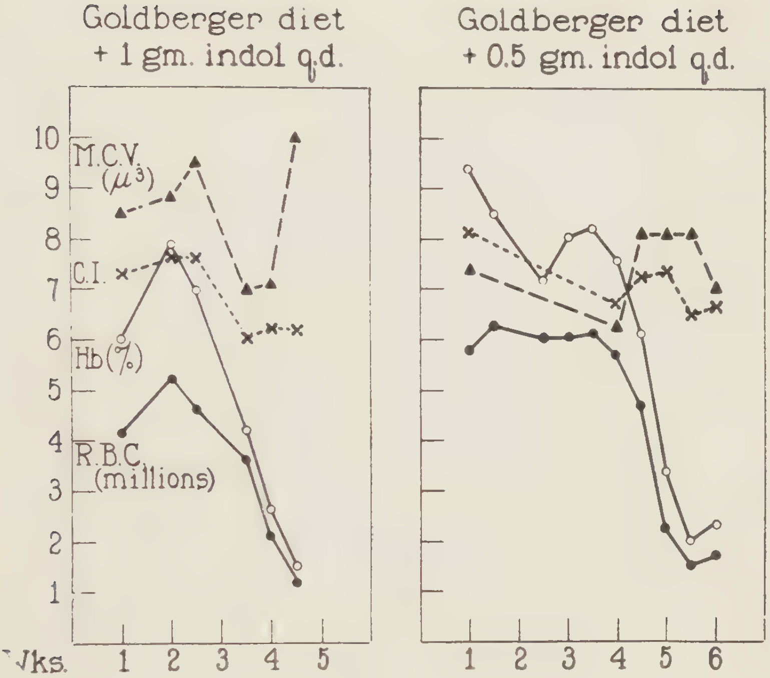
[1] Miller, D. K., and Rhoads, C. P., Proc. Soc. Exp. Biol. and Med. In press.

[2] Houssay, B. A., *Am. J. Med. Sci.*, 1936, **192**, 615.

[3] Miller, D. K., and Rhoads, C. P., *J. Exp. Med.*, 1935, **61**, 173.



Goldberger diet + 1 gm. indol q.d.

Goldberger diet + 0.5 gm. indol q.d.

FIG. 1.

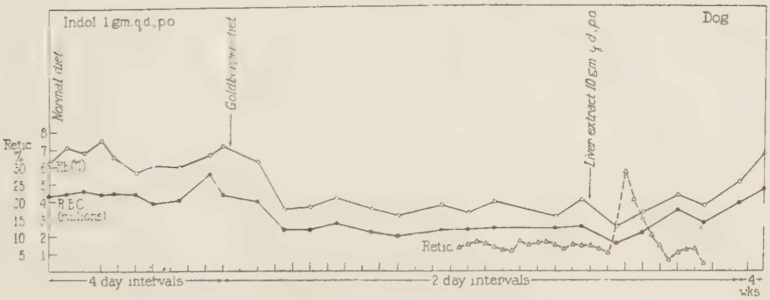


FIG. 2.

the tolerance of the experimental animal to orally administered indol. The drug was fed throughout the study. During a suitable control period, the diet was normal. An irregular mild fall in blood levels occasionally resulted, but was temporary and was overcome as soon as the animal could increase hematopoiesis to compensate for the increased demand. Rare animals were encountered which showed a greater susceptibility to the drug than the average dog. In such instances the dose was adjusted so that essentially normal blood levels were maintained during the control periods. When the blood became stabilized, the diet was changed to one which regularly caused black tongue. A slow but steady fall in blood levels resulted, accompanied at first by an increased number of circulating reticulocytes, followed by slight increase in the blood levels. As the experiment continued, however, anemia became severe and the reticulocytes stabilized at low levels. Goldberger[4] had shown that liver extract was capable of preventing and curing the symptoms of experimental black tongue. Accordingly the effect of administering liver extract to dogs with the indol-and-deficient-diet anemia was tested. A sharp rise in reticulocytes and improvement in blood levels resulted in every instance in spite of continued indol feeding.

Although ample control experiments had shown that no such anemia ever occurred under the experimental conditions due to the diet feeding alone, further experiments were made to control this factor. Dogs fed diets of milk exclusively showed a similar susceptibility to indol. This was equally true of animals which refused food and became voluntarily malnourished.

Pathological studies showed a very marked extension of active marrow during the anemic phase. The marrow throughout was solid, deep red, gelatinous tissue which showed on histological examination an increased cellularity and a predominance of immature forms.

Summary and Conclusion. Orally administered indol is causative of anemia in dogs fed a diet which is causative of black tongue. The same amount of indol does not cause considerable anemia in dogs fed a normal diet. Liver extract is preventive and curative of the anemia.

9347 P

Effect of Diet on Susceptibility of Canine Hematopoietic System to Damage by Amidopyrine.

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From the Hospital of the Rockefeller Institute for Medical Research, New York City.

The association of amidopyrine administration with the occurrence of acute agranulocytosis of human beings has been described by Kracke and Parker,[1] Madison and Squier,[2] and many others.

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[4] Goldberger, J., and Sebrell, W. H., *Pub. Health Rep.*, 1930, **45**, 3064.

[1] Kracke, R. R., and Parker, F. P., *J. Lab. Clin. Med.*, 1933-34, **19**, 799.

[2] Madison, F. W., and Squier, T. L., *J. Am. Med. Assn.*, 1934, **102**, 755.

The attempts to induce hematological disorders in normal animals by the administration of amidopyrine have not been uniformly successful.

Miller and Rhoads[3] reported leukopenia in the terminal phase of acute canine black-tongue and Day[4] reported a leukopenia in monkeys fed diets low in their content of vitamin B_2 (G). This observation suggested that the combination of amidopyrine administration with the feeding of a deficient diet might be productive of abnormal hematopoiesis. Such an experiment has been made but paradoxically the effect observed was anemia rather than leukopenia.

The black-tongue diet fed was that designated by Goldberger[5] as No. 123. Its composition has been described previously.[3] In an extensive series of experiments black-tongue has never resulted from the feeding of this diet in less than 5 weeks nor more than 10. The normal diet fed was a mixture of cooked beef, bread, and dog biscuit, which is known to be capable of maintaining dogs in good health over a period of several years. The amidopyrine employed was lot 1433, manufactured by the H. A. Metz Laboratories. A 5% solution in water was effected by gentle heating. The animals were bled twice weekly from the jugular veins. The blood was collected in bottles containing potassium oxalate. Hemoglobin values were estimated by the Sahli method, using calibrated tubes and standards.

The report includes observations of 17 dogs divided into 4 groups.

Experiment 1. Six dogs were fed the black-tongue diet and 0.5 gm. of amidopyrine was administered daily. All of these animals showed pronounced anemia after periods ranging from 8 to 35 days. When the amidopyrine was discontinued, although the black-tongue diet was still fed, the blood levels returned to normal.

Experiment 2. Three dogs were fed a normal diet and received 0.5 gm. of amidopyrine daily. This series served to control Experiment 1. In no instance was anemia observed.

Experiment 3. In this series 3 dogs were fed the black-tongue diet and 2.0 gm. of amidopyrine were administered daily. Severe anemia occurred in all 3; one animal died after a convulsion and in the remaining 2 the drug was discontinued and recovery ensued.

Experiment 4. This group, composed of 5 animals, tested the effect of the administration of 2.0 gm. of amidopyrine daily while a normal diet was fed. In every instance a well-defined decrease of erythrocyte and hemoglobin levels occurred. Two animals died, one of a pneumonitis and one after a convulsion. The remaining animals recovered after the amidopyrine was discontinued.

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[3] Miller, D. K., and Rhoads, C. P., *J. Exp. Med.*, 1933, **58**, 585.

[4] Day, P. L., Langston, W. C., and Shukers, C. F., *J. Nutrition*, 1935, **9**, 637.

[5] Goldberger, J., and Wheeler, G. A., *Bull. Hyg. Lab. U. S. P. N. S.*, No. 120, 1920, 7.

Specimens of tibial bone marrow were removed at autopsy of the animals which died at the height of the anemia. The marrows were cellular but the number of adult forms of both red and white cells was distinctly less than normal, suggesting an inhibition of maturation. Necrosis was not observed.

Goldberger[5] has reported that the oral administration of yeast is effective in preventing the symptoms of black-tongue and has suggested that the disease is due to a lack in the diet of a vitamin which is contained in yeast. This observation suggested that yeast might be preventive also of the anemia produced by amidopyrine administered coincidentally with the diet feeding.

Experiment 5. Two dogs were fed the black-tongue diet and at the same time 0.5 gm. of amidopyrine was administered daily by stomach tube. When a severe degree of anemia had resulted 10 gm. of Vegex, a commercial yeast autolysate, were administered daily. This was followed by a sharp rise in leukocytes, in erythrocytes, and in hemoglobin The treatment with yeast was given daily for 20 days and then was discontinued, all other factors remaining unchanged.

Following the omission of yeast a slow progressive fall in blood levels occurred. Yeast was administered a second time and on this occasion the reticulocytes were counted. The institution of treatment was followed by an increase in the number of reticulocytes to 10 and 14% respectively and a rise once more in the levels of red and white cells and of hemoglobin.

To substantiate further the conclusion that the anemia resulting from amidopyrine administration was due to the lack of some constituent of yeast, 2 dogs were fed the diet plus 10 gm. of autolyzed yeast daily. At the same time 0.5 gm. of amidopyrine was administered daily by stomach tube. No anemia occurred during the 2-month period of observation, although a fall of blood levels occurred in every dog so treated without the supplement of yeast.

Summary and Conclusions. The daily administration of 0.5 gm. of amidopyrine causes anemia in dogs fed a diet which causes canine black-tongue. No anemia results when a normal diet is fed. The anemia is associated with a suppression of maturation of hematopoietic bone marrow cells and it may be prevented and cured by supplementing the diet with autolyzed yeast.

9348

Liver Extract and Reticulocytosis in the Guinea Pig.

L. E. Hummel. (Introduced by C. P. Rhoads.)

From the Hospital of the Rockefeller Institute for Medical Research, New York City.

Jacobson[1] has reported that an increase in the number of circulating reticulocytes follows the intraperitoneal injection in the guinea pig of liver extract which is curative of pernicious anemia. The results of this test were stated to be sufficiently accurate to differentiate not only between potent and non-potent liver extracts, but even to quantitate the antipernicious effect of various dilutions of the same extract. Goodman, Geiger, and Klumpp[2] have failed to confirm this observation. They stated that guinea pigs showed an irregular reticulocytosis without treatment and that no response to the injection of antianemia substances occurred.

Miller and Rhoads[3] published studies based upon Jacobson's method and concluded that an increase of guinea pig reticulocytes followed the feeding of various antianemia substances. This method avoided the possible non-specific reticulocytogenic effect of the intraperitoneal injections of irritant substances.

In view of the very striking discrepancy between the experimental results of Jacobson and of Goodman and his coworkers it was thought advisable to reinvestigate the subject in an attempt to obtain an answer to the following 5 questions:

1. Do spontaneous variations in the number of circulating reticulocytes occur in guinea pigs maintained under absolutely constant conditions? 2. If spontaneous variations occur are they of such a degree and frequency as to serve as a valid objection to the employment of the guinea pig as a test animal? 3. Does the guinea pig with otherwise constant reticulocyte levels react to the oral administration of potent liver extract by a reticulocytosis? 4. Does the oral administration of the same liver extract after inactivation by heat provoke similar responses? 5. Do factors other than the administration of liver extract cause reticulocytosis in the guinea pig?

Experiments bearing on these 5 fundamental points have been made. The percentage of reticulocytes of normal guinea pigs, kept under standard conditions, have been followed daily for 6 months. The effects of changes in diet, of liver extract feeding, and of feeding liver extract after inactivation of the anti-anemia factor have been studied.

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[1] Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 665.

[2] Goodman, L. S., Geiger, A. J., and Klumpp, T. G., *J. Clin. Invest.*, 1936, **15**, 435.

[3] Miller, D. K., and Rhoads, C. P., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 419.

Nineteen adult white guinea pigs of the same strain were kept under absolutely the same conditions of room, cages, bedding, food, and handling as far as was possible. Reticulocytes were counted by the dry-smear method. The animals were bled by pricking a minute ear vein. The greatest care was observed to allow no more than 0.05 cc. of blood to escape. Smears were made by the usual method on cover slips upon which a film of brilliant cresyl blue had been dried. Many check counts gave very few divergent results. As a source of anti-anemia substance, liver extract, Lilly N.N.R., was employed in a 50% solution in water. It was fed from a pipette. Inactivated liver extract was prepared according to the method of Goodman by heating to 200° for 2 hours in a chemical oven.

During the first 2 weeks of observation the levels of reticulocytes varied considerably without treatment and in a number of the animals reached heights which would be considered positive response to therapy according to the criteria of Jacobson. The diet was changed after 17 days of study after the reticulocytes had been stable for 7 days. At this point 2 guinea pigs had been discarded because of intercurrent disease. In 9 of the remaining animals a distinct reticu-

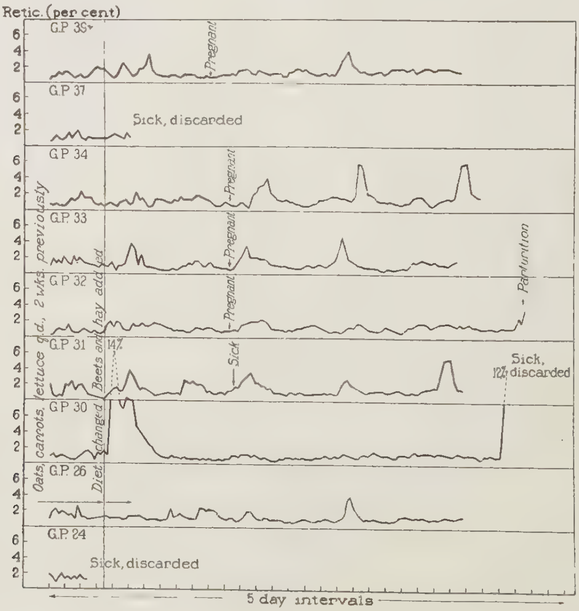

FIG. 1.

locytosis occurred following the change in diet, whereas in 8 no such effect was observed. During the next 3 months 7 more guinea pigs were discarded, 4 because of pregnancy with reticulocytosis, 2 with intercurrent disease and reticulocytosis, and 1 with reticulocytosis without apparent cause. (Fig. 1.)

Experiment I. (Fig. 2.) From the original group 10 animals remained which had at no time during 3 months of constant observation shown any increase in reticulocytes to levels of 2% or over for a period of 2 successive days. When these 10 guinea pigs were fed 0.6 gm. daily of liver extract, Lilly, every animal showed an increase in numbers of reticulocytes which was sustained, was well above the 2% level, which came within the time limits of a reticulocyte rise in human pernicious anemia, and which showed a so-called physiological curve with a rise and then a fall. Such consistent results would seem to be wholly outside of the range of either spontaneous rises or of experimental error.

Experiment II. (Fig. 2.) The 10 animals which had responded by reticulocytosis to the feeding of liver extract were fed the same amount of liver extract after inactivation by charring according to the method of Klumpp. No increase of reticulocytes was observed.

Experiment III. After a failure to respond to inactivated liver

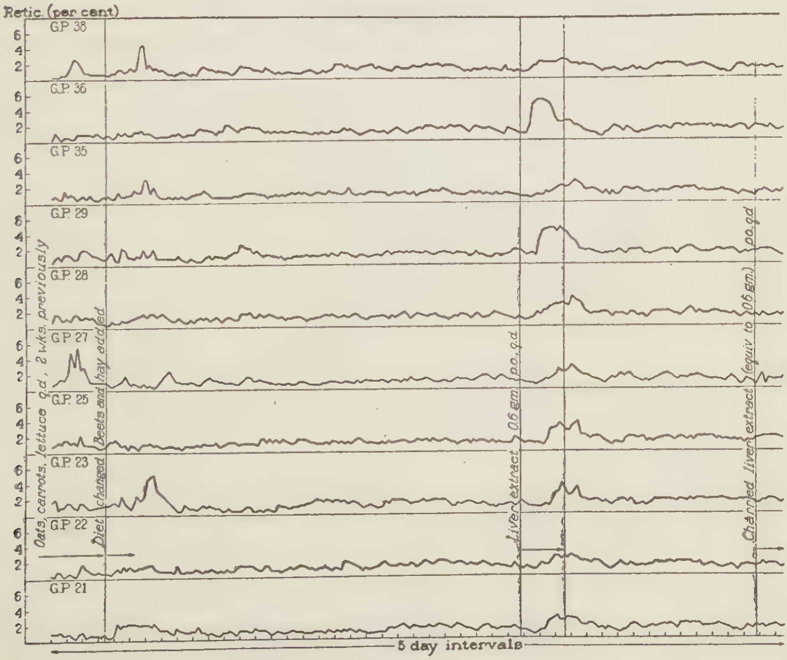

FIG. 2.

extract the same animals were retested by feeding once more liver extract of known potency. For a second time all the animals responded with a reticulocyte peak although not so dramatically as after the first test.

From the experiments presented it appears that increases in reticulocyte numbers can follow a variety of events. Changes in dietary, pregnancy, injury, and infection all seem to exert an effect. Jacobson[4] has shown that excessive loss of blood may also be productive of rises. Lastly, rises do take place rarely, which are spontaneous as far as can be ascertained. It is also clear that animals can be selected which show no serious variation in reticulocyte numbers over a 3-month control period under absolutely constant conditions. These same animals will respond to the oral administration of liver extract by a rise in reticulocytes and fail to respond to inactivated liver extract.

When these experiments are compared to those of Jacobson and of Goodman, two sharp differences are immediately apparent. Our animals were tested by the oral administration of liver extract, both active and inactivated. This route of administration was selected in order to avoid the possible error inherent in any method which depends upon the injection of a known irritant substance. Any error which might occur from the use of the oral as opposed to the parenteral route should clearly be on the side of failure to respond rather than of false positive responses.

The second discrepancy between the results of our experiments and those of other workers concerns the use of very long control periods (3 months) and the rigid exclusion of any animals which evince a rise of reticulocytes from whatever cause during those periods. From the rarity of spontaneous rises in our experiments it is clear that they should not be a serious factor in the interpretation of results from animals which are kept under absolutely standard and constant conditions. Our experiments cannot be compared justly to those of either Goodman or Jacobson however, since in the studies of both of them short control periods were employed; hence, periods of handling and bleeding alternated with periods of rest. In such a delicate mechanism as the maintenance of reticulocyte levels it is inevitable that such factors could easily increase the numbers of reticulocytes by one per 100, sufficient to give a response by Jacobson's criteria.

It is not the purpose of these experiments to answer the question of whether or not the guinea-pig response is due to the administration of that substance which is effective in the induction of remissions in pernicious anemia of human beings. Their only aim is to show that under proper conditions reticulocytes may remain at constant levels and that rises occur when liver extract is fed. Liver extract is a complicated and little-understood substance. It is rich in the vitamin B complex as well as in the anti-anemia factor. The question of which constituent is effective in the guinea pig must await further investigation.

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[4] Jacobson, B. M. Personal communications.

Summary and Conclusions. 1. The number of circulating reticulocytes in the guinea pig increases after a variety of physiological disturbances. 2. By suitable care in handling and feeding stable reticulocyte levels may be maintained for as long as 3 months. 3. Rises of reticulocytes follow the feeding of liver extract. 4. Similar rises do not follow the feeding of charred liver extract.

9349 P

Effect of Colchicine on Human Tumors.

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The action of colchicine upon division of normal and malignant cells has been studied by several investigators. Dustin[1] described a remarkable increase in the number of mitoses in malignant growths in animals following the injection of colchicine. Ludford[2] found that colchicine applied to cultures of normal and malignant cells of animals brought about an arrest of mitosis for several hours, and that prolonged application destroyed the cells held up in division while the resting cells still survived, and that a single injection in animals produced an arrest of mitosis for several hours. The mitoses were arrested in the metaphase, which Ludford believes is due to the failure of the mitotic spindle to form and function in the normal manner. He also found that the largest accumulation of arrested mitoses occurred in tissue in which cell division is normally of frequent occurrence. This phenomenon has been utilized by others[3, 4] as an index of the rate of cell growth. It, therefore, seemed that the method might prove of value in studying the growth characteristics of human tumors.

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[1] Dustin, A. P., *Bull. Acad. Med. Belg.*, 1934^2, **14**, 487.

[2] Ludford, R. J., *Arch. f. exper. Zellforsch.*, 1936, **28**, 411.

[3] Brues, A. M., *J. Physiol.*, 1936, **86**, 63.

[4] Allen, Edgar, Smith, G. M., and Gardner, W. U., *Endocrinology.* In press.

The colchicine effect on tumors was studied in a group of 21 patients who received the drug either subcutaneously or intramuscularly. Fifteen of the 21 patients had control biopsies before receiving the colchicine and of this group 11 showed definite arrest of mitosis in the metaphase. A biopsy was performed, when possible, to establish the diagnosis, and to serve as a control. Colchicine was then administered, and in most instances a second biopsy was performed or the entire tumor removed 9½ hours later. In certain inaccessible tumors it was impossible to perform a biopsy before the removal of the tumor. However, the colchicine effect could be observed and compared with similar tumors.

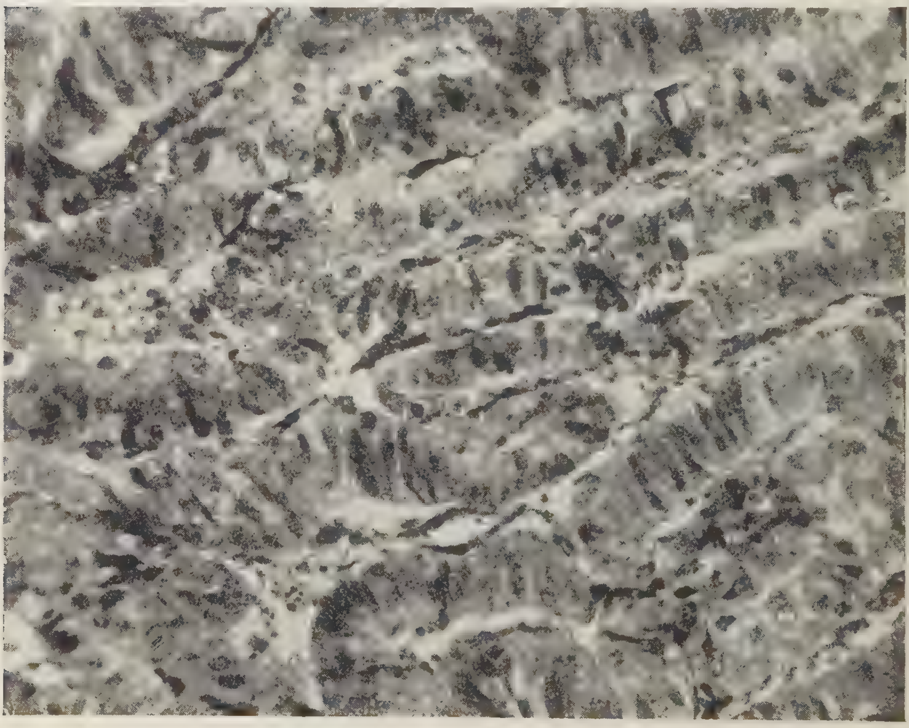

FIG. 1.
Adenocarcinoma of rectum. H&E. 200 ×. Before colchicine.

In some cases, such as the one here reported, biopsies were taken at intervals varying from 4 to 18 hours after injection. The drug was diluted in saline solution, and administered either intramuscularly or subcutaneously. Doses varying from one to 4 mg. were used, and as little as 1½ mg. has produced a typical colchicine effect in some tumors.

The following case history is given as an example of the results obtained. A 50-year-old white male (A-67935) was operated on for a carcinoma of the sigmoid colon. The tumor was delivered on the abdominal wall according to the Mikulicz technique with its blood supply intact, and was readily accessible for biopsy. A portion of the tumor was removed as a control, following which 4 mg. of colchicine were administered subcutaneously. Biopsy of the tumor was performed at 5 and 9½ hours after the injection of the drug, and at 12 hours the entire tumor and adjacent bowel were excised. The microscopic preparation revealed an anaplastic adenocarcinoma Grade III (Fig. 1). The biopsy before colchicine injection showed all phases of mitosis, whereas following colchicine almost all mitoses were in the metaphase. The mitotic figures present in 20 oil immersion fields selected at random in each biopsy were counted with the following results:

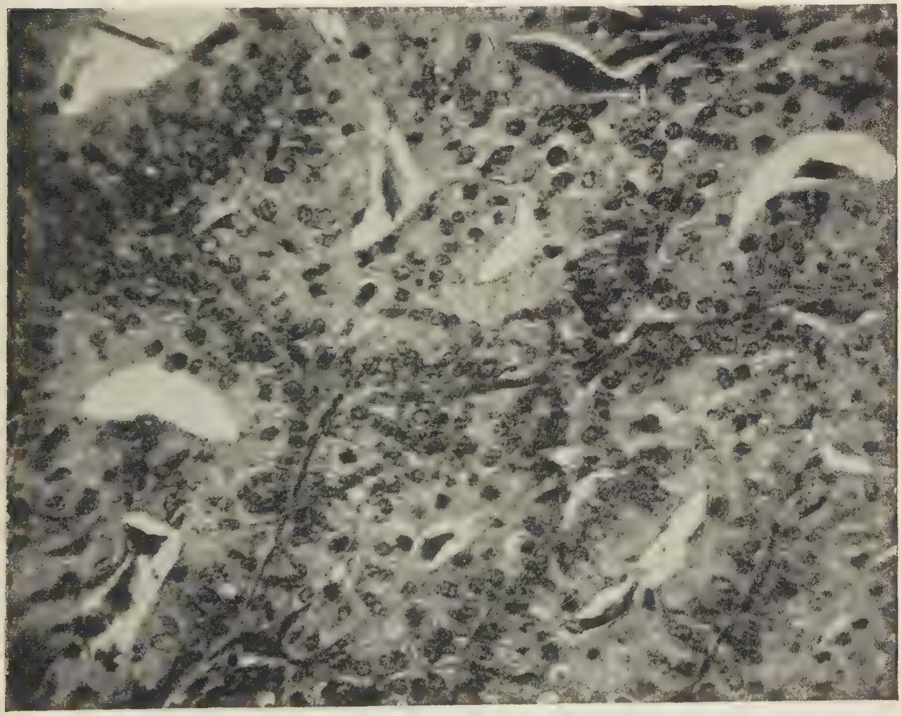

FIG. 2.
Same tumor. H&E. 200 ×. 12 hours after 4 mg. colchicine.

Control				Average	2.6	per	high	power	field
5	hr.	after	injection	”	7.3	”	”	”	”
9½	”	”	”	”	12.0	”	”	”	”
12	”	”	”	”	19.6	”	”	”	”

The number of mitotic figures was still increasing up to 12 hours, at which time there was an increase of over 700%. In some fields in the 12-hour specimen as many as 50% of the cells were in mitosis.

The accumulation of mitotic figures after colchicine cannot be attributed to delivery of the tumor on the abdominal wall, since control biopsies taken before and 10 days after the Mikulicz procedure showed approximately the same number of cells in mitosis per high power field. The repeated biopsies are probably not responsible for the changes noted, as similar accumulations of mitoses after colchicine have been observed in other intestinal tumors that were not subjected to multiple biopsies. It should be emphasized that the 12-hour specimen represents the entire tumor and a large portion of adjacent bowel. The sections of this tumor far removed from the sites of the small biopsy taken at the earlier period, as well as the normal mucosa beyond the tumor mass, showed the same striking accumulation of mitoses.

The subcutaneous and intramuscular injection of colchicine in the doses used has not been accompanied by any general toxic symptoms. Many patients, however, showed evidence of local irritation manifested by local soreness and redness, and in some instances by paresthesia. While these symptoms all disappeared spontaneously, nevertheless the discomfort in some patients was of such degree as to make it desirable to use some other method for administering the drug. At present, studies are being carried out on the effect of the drug by mouth.

There is insufficient data, owing to the small number of any one type of tumor, to permit us to draw conclusions as to its effect on tumors in general. It is, however, apparent that the colchicine technique makes it possible in some instances to obtain a more accurate index of the rate of growth of the tumor than can be obtained by ordinary methods.

9350 P

Effect of Cortin on High Blood Non-Protein Nitrogen of Partially Nephrectomized Rabbits.*

MARVIN H. KUIZENGA. (Introduced by S. Tashiro.)

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By limitation of the kidney tissue to a minimum compatible with life, blood non-protein nitrogen levels considerably higher than normal can be maintained for periods of several weeks or even months. The purpose of this paper is to report the effect of cortin, made by the method of Swingle and Pfiffner, on the blood non-protein nitrogen of partial renal insufficiency. Adult rabbits of both sexes were used in these experiments. The diet consisted of alfalfa hay plus vegetable greens twice weekly. The rabbits were fasted 12 to 16 hours before the beginning of an experiment. The cortin was administered intraperitoneally and the blood samples withdrawn from the marginal ear vein. The technique for partial nephrectomy described by Bradford[1] was used. A wedge shaped piece of kidney tissue was first removed from one kidney and after recovery from this operation the other kidney was entirely removed. The percentage of kidney tissue removed was calculated upon the basis that the total kidney tissue was twice that of the kidney removed at the second operation.

In a series of 22 rabbits it was found that when the kidney tissue was limited to 30%, the initial increase in the non-protein nitrogen of the blood was followed by several weeks of lower values, still higher than normal, and finally by increases up to 400 mg. per 100 cc. before death. These results confirm those of Anderson.[2]

In Table I are given the blood non-protein nitrogen levels before and after the cortin injections. Rabbit No. 775, from which 72% of kidney had been removed, was allowed to become pregnant and 2 weeks before parturition, the non-protein nitrogen began a steady climb. This was twice interrupted and appreciably lowered by injection of cortin. Before parturition the NPN rose to 95 to 100 mg. per 100 cc. blood, which after delivery of a normal litter of 5, dropped back to 75, where it remained until an injection of 15 cc. of

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* The results here reported were taken from a thesis submitted to a committee of the graduate school of the University of Cincinnati in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1933.

[1] Bradford, J. R., *J. Physiol.*, 1897-1899, **22** and **23**.

[2] Anderson, H., *J. Exp. Med.*, 1924, **39**, 707.

TABLE I.

Rabbit No.	Injections of cortin	Blood non-protein nitrogen per 100 cc. at time of inj., mg.	Lowest non-protein nitrogen within 24 hr. after inj.
439	5 cc. twice. 2nd inj. one hour after 1st	266	250
443	3 cc. twice. 2nd inj. 1½ hr. after 1st	233	162
434	2 cc. three times at 2 hr. intervals	131	111
432	3 cc. twice. 2nd inj. 2 hr. after 1st	88	70
593	6 cc.	163	149
775	6 cc.	74	59
767	10 cc. twice. 2nd inj. 2 hr. after 1st	336	248
758	10 cc.	138	126
757	10 cc. at 10:30 A.M. 5 cc. at 1:00 P.M.	98	59
753	10 cc.	216	188
753	10 cc.	260	183
775	5 cc.	70	55
775	10 cc. at 10:00 A.M. 5 cc. at 1:00 P.M.	75	54

"cortin" preparation lowered it temporarily to 50. Four of the litter of 5 survived and remained healthy.

Controls were made with 0.9% NaCl, the solvent in which the cortin was injected. Two rabbits with blood non-protein nitrogen of 140 and 75 were each given 10 cc. of 0.9% NaCl solution. Samples were taken, as in the cortin injected group, at regular 2-hour intervals and again 24 hours after injection. In both cases no decrease in the non-protein nitrogen values was observed. The former increased to 160 and the latter to 86 mg. %.

The extracts used were tested in young bilaterally adrenalectomized white rats, and were found to be effective both in reviving these animals from adrenal insufficiency and in maintaining their lives.

Anderson[2] reported that pregnancy in the 6th week after nephrectomy in a rabbit which had only 63% of kidney removed resulted in death with blood urea nitrogen of 400 mg. %. He reports that none of the nephrectomized rabbits were successful in raising their litters even though the reduction of kidney was in no case greater than 65%.

It is probable that the lowering of the high blood non-protein nitrogen in partial renal insufficiency by injection of cortin is a result of an effect of the cortical hormone on the remnant of kidney remaining. A relationship of the adrenals to kidney function has been suggested. Marshal and Davis[3] postulated a decreased kidney function after adrenalectomy, since urea and creatinine injected into the blood were excreted very slowly. Banting and Gairns[4] described hyperemia of the kidney with swelling of the tubules, and found albuminous casts and blood cells in the tubules. Swingle[5] found albumin in the urine of animals showing marked symptoms of adrenal insufficiency and reported congestion and hemorrhage in the kidneys. Hartman, *et al.*,[6] reported an accumulation of large quantities of lipoid substance in the *tubuli contorti* of the kidney.

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[3] Marshal and Davis, *J. Pharm. Exp. Therap.*, 1916, **8**, 525.

[4] Banting and Gairns, *Am. J. Physiol.*, 1926, **77**, 108.

9351

The Blood Picture of the Normal Dog.

H. D. BRUNER AND G. E. WAKERLIN.

From the Department of Physiology and Pharmacology, University of Louisville School of Medicine.

Since Wintrobe, *et al.*,[1] have pointed out the relative paucity of information in the literature concerning normal blood values for the dog, we are briefly recording additional data obtained during certain experimental studies.

Adult mongrel dogs weighing from 6 to 25 kg. were used. They were housed in individual cages and fed a commercial dog food supplemented with bones. Blood was drawn from the saphenous vein at approximately the same hour of the morning and collected into heparin. The blood values determined were the red blood cell count, hemoglobin, volume of packed red cells, reticulocyte percentage, and leucocyte count. Each red cell count was the average of 2 counts from separate pipettes differing by less than 200,000 cells per cmm.* Hemoglobins were determined by the Newcomer method. The volume of packed erythrocytes was the average of readings from 2 Wintrobe hematocrit tubes.[2] Reticulocytes were recorded from a count of 1000 red cells by the wet mount technique.[3] Each leucocyte count was obtained from the average of 8 sq. mm.* Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were calculated.[4] A total of 251 weekly counts was made on 34 dogs, 17 males and 17 females, during a maximum period of 11 months.

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[5] Swingle, W. W., *Am. J. Physiol.*, 1928, **86**, 450.

[6] Hartman, F. A., MacArthur, C. G., Gunn, F. D., Hartman, W. E., and MacDonald, J. J., *Am. J. Physiol.*, 1927, **81**, 244.

[1] Wintrobe, M. M., Shumacker, H. B., and Schmidt, W. J., *Am. J. Physiol.*, 1935, **114**, 502.

* The hemacytometers and red and white cell pipettes were certified by the United States Bureau of Standards.

[2] Wintrobe, M. M., *Am. J. Med. Sci.*, 1933, **185**, 58.

[3] Wakerlin, G. E., Bruner, H. D., and Kinsman, J. M., *J. Pharm. and Exp. Therap.*, 1936, **58**, 1.

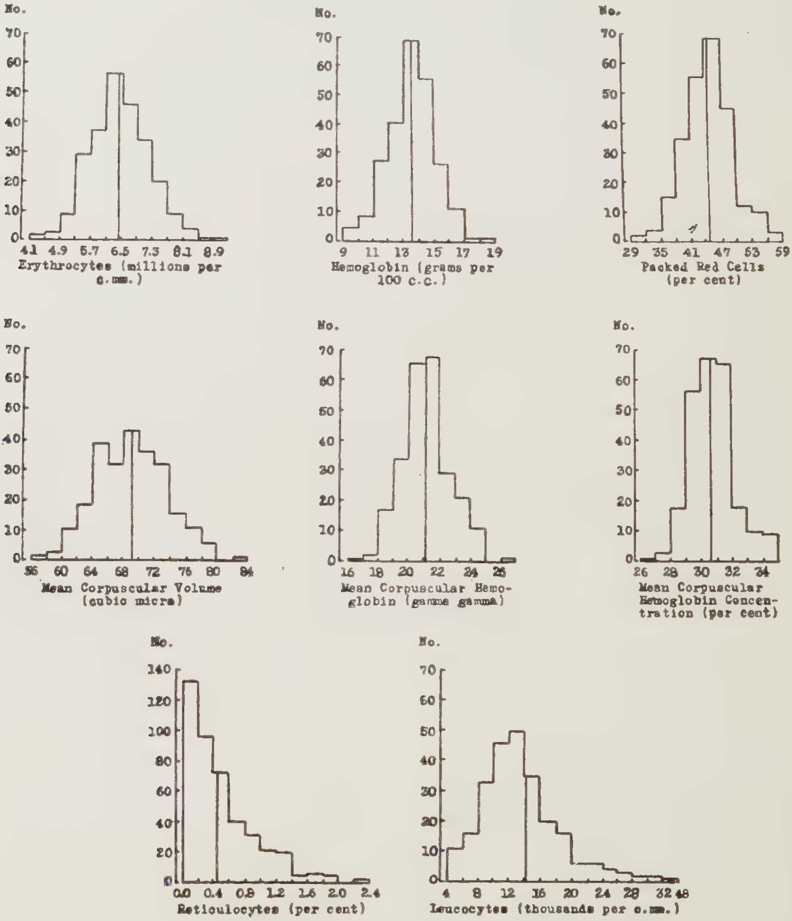

FIG. 1.

Results of 251 Blood Examinations on 34 Normal Dogs.

The results are recorded in the histograms of Fig. 1 and in the statistical analysis of the findings in Table I. We noted, as have others, that the erythrocyte count of the dog may vary as much as 1.5 millions within 7 days and that variations of 1.0 million were not unusual. These fluctuations in red cell count were accompanied by more or less corresponding changes in the hemoglobin and hematocrit values. The mean corpuscular volume, mean corpuscular

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[4] Wintrobe, M. M., *J. Lab. and Clin. Med.*, 1932, **17**, 899.

TABLE I.
Statistical Analysis of 251 Blood Counts on 34 Normal Dogs.

	Mean	Standard Deviation	Coefficient of Variation (%)	Calculated Extremes		No. Observations beyond Calculated Extremes	
				Maximum	Minimum	Greater	Less
Erythrocytes (millions per cmm.)	6.45±.03	0.76±.02	11.73	8.73	4.17	1	2
Hemoglobin (gm. per 100 cc.)	13.56±.07	1.60±.05	11.84	18.36	8.76	1	0
Hematocrit (%)	44.28±.20	4.78±.14	10.79	58.62	29.94	0	0
Mean Corpuscular Volume (cubic micra)	68.9±.19	4.53±.14	6.58	82.49	55.31	1	0
Mean Corpuscular Hemoglobin (gamma gamma)	21.1±.07	1.57±.05	7.44	25.81	16.39	1	0
Mean Corpuscular Hemoglobin Concentration (%)	30.7±.06	1.40±.04	4.56	34.90	26.50	1	0
Reticulocytes (%)*	0.44±.014	0.42±.010	95.7	1.70	0.0	7	—
Leucocytes (thousands per cmm.)	14.18±.22	5.21±.16	36.75	29.81	0.0	2	—

*420 counts.

hemoglobin, and mean corpuscular hemoglobin concentration may vary as much as three times the standard deviation within 7 days. The mean reticulocyte value is comparable to that of Krumbhaar.[5] The mean value and the wide variations in the number of leucocytes compare favorably with the findings of Mayerson[6] and the compilation of Scarborough.[7] Our statistical analysis agrees closely with that of Wintrobe.[1] In agreement with previous reports for the dog,[1] we noted no significant differences between the erythrocyte count, hemoglobin, volume of packed red cells, reticulocyte percentage, and leucocyte count of the male and female dogs studied.

Summary. The results of 251 determinations of the red blood cell count, hemoglobin, volume of packed red cells, mean corpuscular volume, hemoglobin, and hemoglobin concentration, reticulocyte percentage and leucocyte count of 34 normal dogs are recorded. No significant sex differences for these values were observed.

9352 P

Study of Peripheral Circulation by Means of an Alternating Current Bridge.

HUBERT MANN.

When the electrical conductivity of any part of the body is measured by means of an alternating current bridge it is found that this conductivity shows a rhythmic variation synchronous with the pulse. This phenomenom which can be observed in any accessible part of the body, offers a method of studying the peripheral circulation. For experimental purposes a single finger, generally the right index finger, has been chosen as the subject of this investigation.

The experimental set-up is as follows: 2 strips of cotton gauze or thin metal foil are applied to the finger after moistening with a salt solution or an electrically conducting paste or jelly, care being taken that the gauze or foil does not constrict the finger or impede the circulation. One of these electrodes is placed near the tip and the other near the base of the finger. Through these strips the finger is then connected to an alternating current bridge. This bridge, instead of having the customary earphones, is connected to an amplifier and the output of the amplifier after rectification actuates a recording galvanometer. A change in the electrical conductivity of the finger is thus recorded.

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[5] Krumbhaar, E. B., *J. Lab. and Clin. Med.*, 1922, **8**, 11.

[6] Mayerson, H. S., *Anat. Rec.*, 1930, **47**, 239.

[7] Scarborough, R., *Yale J. Biol. and Med.*, 1930, **3**, 359.

Care is taken that the patient's hand and arm are in a comfortable position with the fingers relaxed and free from constriction or any discomfort. During the course of the experiment a current of about one volt and one milliampere passes through the right index finger. This current does not give rise to any sensation or to any objective changes even after several hours' continuous application.

The curves obtained are not due to variation in contact resistance between the finger and the electrode. This is evident from the fact that when the electrodes are made of gauze soaked in salt solution and applied loosely to the finger, the same curve is obtained as when the electrodes are made of metal foil. Variations in the electrical conductivity of the finger, as recorded by this instrument, seem to be due solely to circulatory factors. When the circulation in the finger is stopped by a blood pressure cuff on the upper arm the instrument then records a level base line without rhythmic variations (Fig. 1). If the blood pressure cuff is deflated slowly the instrument begins to record small waves when systolic pressure is reached and these waves increase in amplitude, reaching their maximum when the pressure in the cuff approaches the diastolic blood pressure.

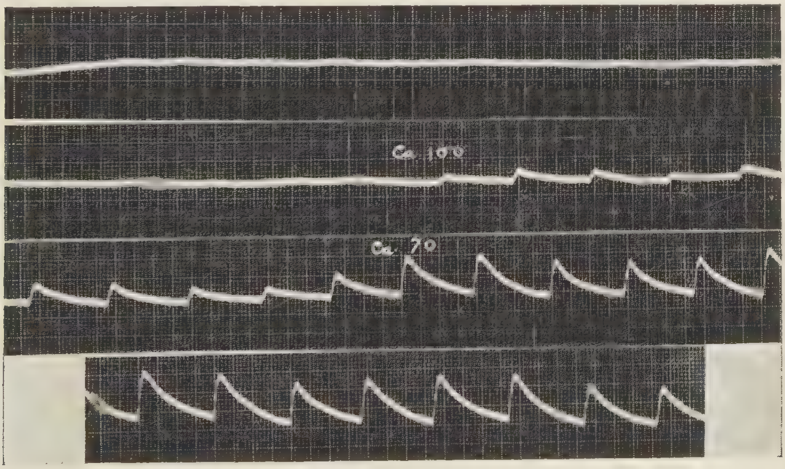

Fig. 1.

Continuous record taken from the right index finger while a blood pressure cuff was attached to the right upper arm. The pressure in the cuff was gradually lowered. The curve begins to appear as a small rhythmic wave when the pressure in the cuff reaches systolic blood pressure and the curve attains its maximum height when the pressure in the cuff approximates diastolic blood pressure. An upstroke in the curve indicates decreased impedance in the finger.

While it is not safe to assume that the height of the recorded curve is directly proportional to the blood flow through the finger, it seems obvious that variation of the blood flow through the finger produces variation in the recorded curves, and that there is a positive correlation between the height of the recorded curve and the volume of the peripheral circulation.

This correlation is evident in experiments with drugs whose peripheral circulatory action is well known. Nitroglycerin: 1/100 grain under the tongue: produced a rapid, marked increase in the height of the curve in all 5 individuals tested. Smoking had the opposite effect. Ergotamine tartrate: 1 mg. injected intramuscularly: produced a gradual decrease in the amplitude of the curve, the maximum effect occurring about one hour after injection. Fig. 2 illustrates some of the characteristic curves.

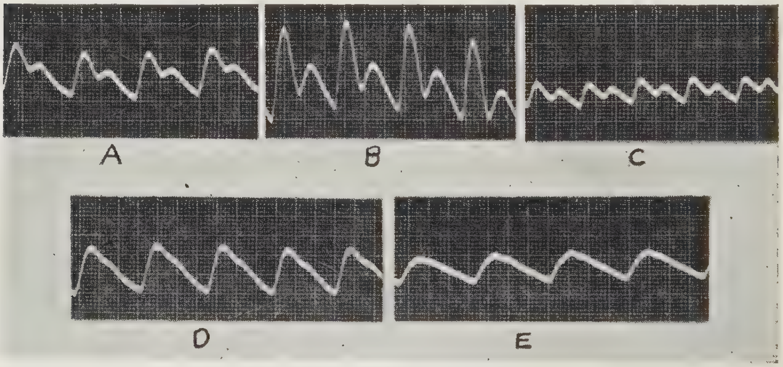

Fig. 2.

A— shows a curve taken from the right index finger of a normal adult. B— is a record from the same finger 10 minutes after 1/100 mg. of nitroglycerine under the tongue. C— is a record of the same patient immediately after smoking the first morning cigarette. D— is a record taken from the right index finger of a normal adult. E— is a record of the same finger 50 minutes after the intramuscular injection of 1 mg. of ergotamine tartrate.

It will be noted that the curves in Fig. 2 show not only changes in height but also changes in shape. Curves A and D were both made from the right index fingers of apparently normal adults and, although of about the same height, show very different shapes. Curve B shows a characteristic shape which is produced regularly by nitroglycerine. In all the curves recorded, the change which occurred in the finger with each pulse beat was an increased capacitance and a decreased resistance. Of these 2 effects, the capacitance effect was by far the more pronounced. Because of the complexity of the physical and mechanical elements involved, the evaluation of the significance of the shape of the curves can not as yet be published. Suffice it to say that the phenomena observed are constant and easily reproducible so that the evidence at hand indicates that the electrical changes in the finger provide a new and useful method for studying circulatory changes.

Summary. The alternating current bridge offers a method of studying the peripheral circulation, and of recording changes produced by physiological and pharmacological agents.

9353 P

Leucocytic Infiltration of Irradiated Mouse Sarcoma 180.

Robert Chambers and C. G. Grand.*

From the Department of Biology, Washington Square College, New York University.

In tissue cultures of the Crocker Mouse Sarcoma 180 a pronounced difference was found in the numbers of blood granulocytes which migrated from explanted fragments of tumors irradiated *in vivo, in vitro,* and of untreated controls.

The tissue culture method affords a ready means of detecting the presence of leucocytes in a given tissue. The polymorphonuclear leucocytes are the first cells to migrate from the explant into the surrounding plasma medium and, if present in large numbers, form cloud-like masses extending beyond the periphery of the explant within a few hours after incubation. This method was recently used as one of the means of determining the accumulation of leucocytes in various mouse tumors which had been injected with starch.[1]

The tumors used were growths which had developed for about 10 days after subcutaneous inoculation. Their size varied from about 5 to 8 mm. in gross diameter. Sarcoma 180 is a rapidly growing tumor which spontaneously undergoes partial necrosis. In selecting fragments for tissue culture, care was taken to note whether they were from necrotic or from non-necrotic regions for comparisons with irradiated or non-irradiated specimens.

Studies were also made of a large number of histological sections of tumors removed at hourly intervals after irradiation. These were compared with untreated tumors of similar ages and also with tumors irradiated after extirpation.

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* Aided by the International Cancer Research Foundation, Philadelphia, Pa.

[1] Chambers, R., and Grand, C. G., *Am. J. Cancer,* 1937, **29**, 111.

Irradiation of the tumors by X-rays was done through the courtesy of the Memorial Hospital, New York City, according to the technique recently described by Sugiura.[2] Single dosages were given. They varied between 1500 to 2800 roentgen units of hard X-ray.

Untreated tumors of various ages grown in tissue culture showed very little evidence of outwandering granulocytes irrespective of whether the fragments used were from necrotic or non-necrotic regions. A similar scarcity of typical blood granulocytes was observed in histological sections of the tumors in the younger tumors, showing incipient regions of necrosis, and also in advanced tumors with extensive necrotic regions. In the necrotic regions, which were frequently extensive in the older tumors, there were clumps and scattered granules of hematoxylin-staining material, but very little of it could be definitely identified as blood granulocytes. The same was true for the marginal zones between the necrotic and non-necrotic regions. This lack of identifiable blood granulocytes accorded with the results from the tissue cultures in which only an occasional outwandering granulocyte could be distinguished around the explants of either necrotic or non-necrotic tissue.

Tumors irradiated after extirpation showed a similar scarcity of blood granulocytes.

The tumors irradiated *in vivo* were removed at hourly intervals and then daily, up to 15 days after the treatment. The tumor tissue was then either fixed for sectioning or used for tissue culture.

Very few blood granulocytes were observed in tissue cultures of tumors removed within 6 hours after irradiation *in vivo*. Those of tumors removed at hourly intervals beyond 6 hours showed a progressive increase in outwandering blood granulocytes until, in those removed 24 to 48 hours after treatment, the blood granulocytes appeared about the explant in relatively large numbers. It was also found that the longer the tumors had remained *in situ* after irradiation *in vivo,* the sparser was the outgrowth of the true sarcoma cells. The amount of sarcomatous growth was in inverse proportion to the abundance of blood granulocytes present. In cultures which showed outgrowths, both of granulocytes and of sarcoma cells, the granulocytes were more abundant in zones where the sarcomatous outgrowth was sparse and vice versa.

The increase in numbers of blood granulocytes was also observed in histological sections of the tumors. In tumors removed 12 to 48 hours or later after irradiation the granulocytes appeared in masses irregularly dispersed throughout the tumors in regions where the sarcoma cells were beginning to undergo pycnosis. Typical blood granulocytes were in the extensive necrotic regions, but they were most abundant in zones surrounding the necrotic regions and were also present in spaces between morphologically normal appearing sarcoma cells bordering the necrotic regions. In these necrotic regions could also be observed the irregular, hematoxylin stained material described for the necrotic regions of untreated tumors. The blood granulocytes were present in quantities throughout the degenerative changes of the irradiated tumors.

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[2] Sugiura, K., *Radiology*, 1937, **28**, 162.

Tests to exclude the possibility that the leucocytic response was due to possible infection of the irradiated tumors were made by bacteriological agar slants. These were uniformly sterile. Moreover, the tissue cultures, in which bacterial colonies develop rapidly and very early, were sterile during their maintenance for over a week. The cultures closely resembled those of fragments of starch-injected tumors in which sterile precautions were rigorously maintained.

These findings indicate that irradiation of Sarcoma 180 induces changes which call forth an accumulation of blood granulocytes in the tumor. The question is still open whether the accumulation is the cause of or subsequent to the necrotic changes. The fact remains that granulocytic response and necrosis accompany one another in the irradiated tumors while they do not in the spontaneous necrotic changes of non-radiated tumors of Sarcoma 180.

9354 P

Rôle of Arteries in Peripheral Resistance of Hypertension.

Myron Prinzmetal and Enid Tribe Oppenheimer. (Introduced by B. S. Oppenheimer.)

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It seems established that arterial hypertension must be due to an increased peripheral resistance, but which part of the vascular bed is concerned in this increase is still somewhat uncertain. Evidence on the whole supports the view that it is due to arteriolar constriction, but some workers believe that the larger arteries also become narrowed in hypertensive conditions.[1]

Our observations represent an attempt to ascertain whether or not the larger arteries are involved in the production of the increased resistance of chronic hypertension. It is known that normally the blood pressure in the arterial bed falls gradually from large to small arteries, and that the greatest fall occurs in the region of the arterioles. If the arteriolar resistance should increase, the arterial blood pressure would be increased throughout, in small as well as large arteries, so that the pressure gradient from large to small arteries would either remain substantially unchanged, or become less. If an increased resistance should occur in the arteries themselves, the pressure gradient would tend to become steeper, since the pressure would be raised in the largest arteries, but would be reduced in the smallest.

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[1] Weiss, S., Haynes, F. W., and Shore, R., *Am. Heart J.*, 1936, **11**, 402.

We therefore measured the blood pressure in a large and a small artery of subjects with hypertension, and compared the figures with those obtained from individuals having normal systolic pressures. Blood pressure was determined in the brachial artery by the usual auscultatory method, and in the digital artery by Gaertner's method,[2] whereby, after rendering the finger bloodless, the pressure is noted at which the finger again becomes suffused with blood. This pressure is assumed to be the systolic pressure in the digital artery.

TABLE I.
Average Brachial-Digital Gradients.

Type of Case	No. of Cases	Brachial Syst. Press.	Digital Syst. Press.	Brachial-Digital Gradient in mm.
Normal	25	124	95	29
Hypertensive				
(a) Benign H.T.	32	184	152	32
(b) Secondary H.T. (renal)	10	187	156	31
(c) Malignant H.T.	9	210	186	24
Averages for all Hypertensives	51	188	158	30
Hypotensive	14	98	71	27

From Table I it will be seen that the average brachial to digital pressure gradient in cases of hypertension shows no significant difference from that in subjects with normal or with low blood pressure. The most marked variation is perhaps a slight *reduction* of gradient in the cases of malignant hypertension.

It would seem, therefore, that there is certainly no significant increase in the brachial-digital pressure gradient in chronic hypertension, suggesting that the increased resistance occurs, not in the larger arteries, but mainly in vessels smaller than the digital artery.

This contention is further supported by observations made on a patient with paroxysmal hypertension due to pheochromocytoma. During the hypertensive attacks, easily induced by moderate exercise, large amounts of epinephrin were demonstrated in the blood[3] by the Pissemski method.*[4] This condition was of particular interest to us, since epinephrin is known to cause constriction of arteries as well as of arterioles. Table II shows that during the hypertensive attacks the brachial systolic pressure rose to 280 mm., while the digital pressure, on the contrary, showed a slight fall, resulting in a steep brachial-digital gradient of 150 mm., as contrasted with the 30 mm. gradient of chronic hypertension. The marked contrast between this case, where arterial spasm is admittedly present, and the cases of chronic hypertension suggests that in the latter condition, where there is no increased gradient, there is no constriction of the larger arteries.

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[2] Formijine, P., *Am. Heart J.*, 1934, **10**, 1.

TABLE II.

Type of Case	No. of Cases	Brachial Syst. Press.	Digital Syst. Press.	Brachial-Digital Gradient
Pheochromocytoma				
(a) Resting	1	184	141	43
(b) During attack		280	130	150
(c) After removal of tumor		133	90	43
Obliterative Vascular Disease				
Thromboangiitis oblit.	2	112	43	70
Sclerodactylia	1	107	27	80
Thrombosis of brach. art.	1	104	29	75

In 4 cases also of obliterative vascular disease (Table II), we found that the brachial-digital fall of pressure was much greater than in normal subjects. These cases indicate that obstruction of the arteries, whether due to spasm or to organic changes, causes an increased gradient in the fall of pressure between brachial and digital arteries.

We therefore conclude that in chronic hypertension the increased resistance occurs in vessels smaller than the digital artery.

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[3] Beer, E., King, F. H., and Prinzmetal, M. To be published shortly.

* In this method the fluid to be tested is perfused through the vessels of a denervated rabbit ear. In our case the patient's blood plasma caused vasoconstriction, which was inhibited by ergotoxin.

[4] Pissemski, A., *Pflüger's Arch.*, 1914, **156**, 430.

9355

Para-Aminobenzenesulfonamide Therapy in Experimental Type III Pneumococcal Pneumonia.

FRANK B. COOPER AND PAUL GROSS. (Introduced by Ralph R. Mellon.)

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh.

We have reported[1] the successful treatment of experimental Type III pneumococcal pneumonias in rats by the daily oral administration of *p*-aminobenzenesulfonamide.

The present report deals with additional results obtained by treating 64 rats, suffering from Type III pneumococcal pneumonia, with *p*-aminobenzenesulfonamide.* These results are contrasted with those from a simultaneous series of 37 similarly infected, but untreated, animals.

The infection technic employed by us differs from that of Nungester and Jourdonais.[2] Under sodium amytal anesthesia the trachea was exposed by an incision 1.0 cm. long. A 16-gauge (B. & S.) trocar, 4.5 cm. long, bearing a protruding, pointed stylet, was inserted between the tracheal rings. The stylet was then withdrawn and the trocar gently inserted for a distance of 3.0 to 3.5 cm. A tuberculin-syringe containing a suspension of pneumococci in mucin was attached to the trocar and 0.07 to 0.10 cc. of the suspension injected, after which the trocar was withdrawn and the skin sutured with silk.

The suspension of organisms was prepared by diluting an 18-hour broth culture of our "420" strain of Type III pneumococcus 1000 times in broth and adding powdered gastric mucin (Armour), with constant stirring until a homogeneous, viscid mixture was obtained. No attempt was made to sterilize the mucin or to standardize the viscosity, since each different suspension had its own series of control animals. In 4 groups of rats the culture was diluted 10,000 times.

The animals were divided into 10 groups, which were treated as described under Fig. 1. Treatment consisted of the oral administration of 125 mg. of *p*-aminobenzenesulfonamide (as a 25% suspension in 15% aqueous gum acacia) daily for 14 consecutive days. All survivors in each group were killed with ether on the fifteenth

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[1] Gross, P., and Cooper, F. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 225.

* Kindly supplied by the Winthrop Chemical Co. and Burroughs Wellcome & Co.

[2] Nungester, W. J., and Jourdonais, L. F., *J. Bact.*, 1935, **29**, 34.

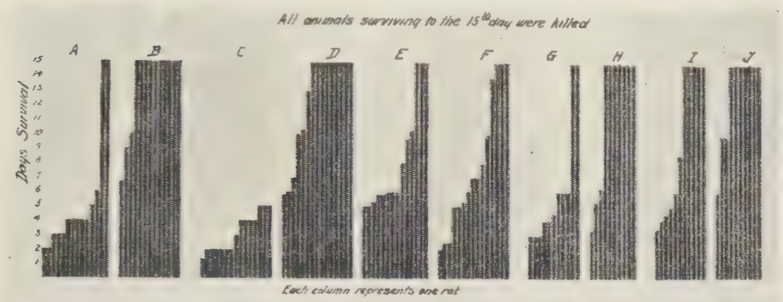

FIG. 1.

A. Fourteen rats, infected with 0.07 cc. of a 1:1000 dilution of an 18-hour broth culture. Untreated controls. (From previous report.[1])

B—Thirteen rats, same infecting dose as Group A, treated immediately after infection. (From previous report.[1])

C. Fifteen rats, infected with 0.1 cc. of a 1:1000 dilution of an 18-hour culture. Untreated controls.

D. Fifteen rats, same infecting dose as Group C, treated after 6 hours.

E. Fourteen rats, same infecting dose as Group C, treated after 30 hours.

F. Fifteen rats, infected in same manner as Group C (except that inoculum had stood for 4 hours in a warm room), treated after 18 hours.

G. Eleven rats, infected with 0.07 cc. of a 1:10,000 dilution of an 18-hour culture. Untreated controls.

H. Ten rats, same infecting dose as Group G, treated after 48 hours.

I. Eleven rats, infected with 0.07 cc. of a 1:10,000 dilution of an 18-hour culture. Untreated controls.

J. Ten rats, same infecting dose as Group I, treated after 24 hours.

day. All animals were necropsied. Smears were made of pleural exudate, peritoneum, and blood from the femoral vein. Sections were made of all lobes of both lungs, of the heart, liver, spleen, and left kidney.

The effect of the treatment on the survival-time of the infected rats is shown graphically in Fig. 1. Group B, treated immediately after infection, showed a 77% survival contrasted with a 14% survival in Group A. Group D, treated 6 hours after infection, had a 60% survival, whereas no animals survived in Group C. Furthermore, the shortest survival-time in the treated group was greater than the longest in the control group. Even in Group E, where treatment was delayed 30 hours after infection, 21% of the rats survived, while all controls (Group C) died within 5 days. Group F, representing an earlier treatment (18-hour interval following infection), but a larger infecting dose, showed practically the same survival-rate, 20%, as Group E. Group H, representing a smaller infecting dose with treatment delayed 48 hours after infection, showed 80% survivals against 18% in Group G. Group J, infected with a dose that allowed a 45% survival-rate in Control Group I, showed a 70% survival when treatment was delayed 24 hours after infection.

The pneumonic consolidation was lobar in distribution, usually involved more than one lobe, sometimes an entire lung, and occasionally both lungs. The animals that succumbed, treated or untreated, showed no consistent difference in the degree of pulmonary involvement. Similarly, no qualitative or quantitative differences could be detected microscopically in the cellular reaction of the treated animals that would distinguish them from the controls.

All smears and sections from both treated and untreated rats revealed a marked infrequency of phagocytic activity; also, they gave no indication that this activity was greater in the treated animals than in the controls.

Empyema, often sufficiently severe to cause the compression of non-pneumonic lobes, was observed in all of the control rats, and 87% of the treated rats that died. Of the fatalities, 26% of the controls showed pneumococci in blood smears, whereas the blood smears of all treated rats were negative. Peritonitis, based on the presence of polymorphonuclear leukocytes and pneumococci in peritoneal smears, was present in 29% of the control fatalities, compared with 8% of the fatalities among the treated animals.

The average survival-time of the rats that succumbed was 3.9 days for the combined control groups, and 6.9 days for the treated groups. Pneumococci were never found in smears of the blood, peritoneum, or pleura, in animals that survived to the fifteenth day. Of all the control rats that survived 15 days, 56% (5 out of 9) showed no grossly demonstrable pneumonia. A similar percentage (56%, 22 out of 39) was found in the treated survivors. Some animals in both series showed pleural adhesions, and all gave microscopic evidence of pneumonitis. The histologic changes in the lungs of the survivors were similar to those described by Gunn and Nungester.[3] Changes in the heart, liver, spleen, and kidney of rats that succumbed were no different in the treated than in the untreated animals.

Treatment resulted in a marked reduction of the mortality-rate of rats infected intrabronchially with virulent Type III pneumococci. This reduction was related to both the interval between infection and initial treatment and the size of the infecting dose.

Of the rats that succumbed to the infection, the average life of those treated was greater than that of the untreated controls. Similarly, there were fewer cases of bacteremia and peritonitis in the treated rats. (This should be qualified to a certain extent since the criterion used to determine the presence or absence of bacteremia is, at best, a very rough one.) Empyema was only slightly less common in the treated animals.

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[3] Gunn, F. D., and Nungester, W. J., *Arch. Path.*, 1936, **21**, 813.

The treatment had no apparent effect upon the type or extent of the pulmonary involvement, which, due to the longer survival-time, was frequently greater in the treated animals than in the controls. The percentage of pneumonias that subsided with a minimum of residue was practically the same in treated and control animals.

Rosenthal[4] found that *p*-aminobenzenesulfonamide was bactericidal for pneumococci of Types I, II, and III *in vitro*. However, since the course and the extent of the pneumonia was not lessened by the therapy, it seems that such bactericidal action was relatively unimportant. Nevertheless, this treatment enabled the host, in many cases, to survive an otherwise fatal toxemia.

Because of these encouraging results, similar therapy was used on human Type III pneumococcal pneumonia. Heintzelman, Hadley, and Mellon[5] report the survival of 7 out of 9 patients treated with *p*-aminobenzenesulfonamide; of 10 cases not treated with this drug only 2 recovered.

Conclusions. *P*-aminobenzenesulfonamide given orally to rats suffering from experimental Type III ("420" strain) pneumococcal pneumonia reduced the mortality and increased the survival-period. The effectiveness of this treatment varied inversely with the interval between infection and initial treatment, and with the magnitude of the infecting dose. The treated rats which succumbed showed less bacteremia and a lower incidence of peritonitis than the untreated ones which succumbed. The course and extent of the pneumonia was not appreciably affected. Treatment apparently enables many of the animals to survive the associated toxemia which is commonly fatal to similarly infected, but untreated, rats.

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[4] Rosenthal, S. M., *Public Health Reports*, 1937, **52**, 192.

[5] Heintzelman, J. H. L., Hadley, P., and Mellon, R. R., *Am. J. Med. Sc.*, 1937, **193**, 759.

9356

Bacteriostatic Effect of Sulfanilamide in Spinal Fluid of Convalescent Cases of Streptococcal Meningitis.

Ralph R. Mellon and Louis L. Bambas.

From the Western Pennsylvania Hospital, Institute of Pathology. Pittsburgh.

During the latter part of August, 1936, and the first week of September, we treated successfully with sulfanilamide what appears to be the first critically studied case on record of streptococcal meningitis. Our second case, one of highest severity, was treated in December. It also recovered. The spinal fluid in each was characterized by a remarkable bacteriostatic effect of the drug, manifesting itself in the first case in 72 hours, and in the second in 48 hours.

In the latter case there were 3,000 white cells per cmm., of spinal fluid, and 8 chains of streptococci per high-power field. One loop of spinal fluid yielded countless numbers of colonies on a blood agar plate; yet after 48 hours' treatment, seeding of ½ cc., of spinal fluid under the same conditions gave no growth, even after 5 days' incubation at 37°C.

When contrasted with the normal serum of a healthy adult, the following results obtain: Both fluids were seeded with 100 streptococci and incubated at 37° for 24 hours. No growth was obtained from the spinal fluid, while the serum showed slightly over 1,000 colonies. A complete clinical report[1] of these cases has been in press for some months.

In a case of brain abscess[2] with a Type V pneumococcus, which was successfully treated, a question arose with the surgeon relative to the accessibility of the spinal fluid to the drug, which was administered para-spinally. A quantitative test for sulfanilamide, devised by one of us (Bambas), proved to be essentially the same as the one later reported by Fuller,[3] except that H acid (1-amino-8-naphthol-3.6-disulfonic acid) was used as the coupling compound.

In 72 hours the patient had received 10 cc. of Prontosil in 2 doses of 5 cc. each, and a total of 40 grains of sulfanilamide in 5 doses. The concentration of the drug in the ventricular fluid was 1:25,000, or 4 mg. per 100 cc. of fluid.

The patient continued to receive 20 grains of sulfanilamide per day for 3 more days. The concentration at this time was 1:35,000 in both blood and spinal fluid, showing the interesting fact that no partition-coefficient between the 2 fluids existed.

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[1] Weinberg, M. H., Mellon, R. R., and Shinn, L. E., *J. Am. Med. Assn.*, 1937, **108**, 1948.

[2] Rowe, N. S., to be published.

[3] Fuller, A. T., *Lancet*, 1937, **232**, 194.

Curiosity as to the mechanism of the bacteriostatic and killing action of the drug led to experiments on its possible effect on the dehydrogenases of the pneumococcus. Sulfanilamide was studied comparatively with other well-known bactericidal agents, and the results follow. We used Type I Neufeld strain, and glucose was employed as a metabolite.

In these experiments the Thunberg technic was employed: one cc. of 1:5,000 methylene blue; one cc. of 0.01 M glucose; one cc. of pH 7.4 phosphate buffer; one cc. of washed cells of Type I Neufeld, mucoid, pneumococcus; and one cc. of the inhibitor were placed in a Thunberg tube. The tubes were exhausted, placed in a water-bath, and the rate of reduction of the methylene blue was noted.

With sodium glycocholate the inhibiting molarity of its concentrations was M/5,000 to M/2,000; with optochin hydrochloride, M/1,500 to M/1,000; with apoquinine hydrochloride, M/750 to M/250; with quinine acetate, M/1,000 to M/200; but with sulfanilamide, M/100 (0.17%). No inhibition of reducing power of Type I Neufeld pneumococcus in the presence of glucose at M/100 (0.17%), the highest concentration obtainable was shown with this technic.

The results show that whereas the ordinary bacteriostatic compounds inhibit the dehydrogenases (a part of the respiratory mechanism), the sulfanilamide does not affect these enzymes. This rules out an effect that the drug might have on the organisms.

9357

A Virus Disease of Swiss Mice Transmissible by Intranasal Inoculation.

A. R. Dochez, K. C. Mills and B. Mulliken.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

Mice have been extensively employed in the study of the virus of human influenza since the discovery by Andrewes, Laidlaw, and Smith,[1] and by Francis[2] that these animals are susceptible to intranasal inoculation of ferret-passed virus. Francis[3] has recently shown that influenza virus cultivated in the chorio-allantoic membrane of the developing chick embryo is pathogenic for mice and does not require ferret passage to raise its virulence. Many of the immunological studies of influenza virus have been made by means of protection tests using mice as test animals. It is obvious, therefore, that if the virus of the common cold could be made virulent for mice, a similar and valuable immunological study of this virus could be made.

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[1] Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2**, 859.

[2] Francis, T., Jr., *Science*, 1934, **80**, 457.

After a number of preliminary failures in attempts to infect mice by intranasal inoculation of the virus of common cold, we began, during the summer of 1936, to use virus for inoculation which was being cultivated in the chorio-allantoic membrane of the chick embryo. By this means, with comparatively little difficulty, 3 different strains of virus were established in mice and could be passed in series by intranasal inoculation of emulsions of infected lung. After the few passages necessary to establish the strains, all 3 produced fairly uniform lesions in the lungs. The mortality rate was much less than that reported for human influenza virus, only about 10% of the mice dying in 4 or 5 days. About 75%, however, showed fairly extensive pulmonary consolidation at autopsy. The areas of pulmonary consolidation were plum colored, often involving a whole lobe, and their gross appearance closely resembled that seen in mice infected with human influenza virus. The serological relationship to one another of the 3 strains of common cold virus was studied by means of protection tests in mice. Because of the relatively low virulence of the virus for mice some difficulties of interpretation were encountered. However, preliminary tests seemed to indicate that the 3 strains of cold virus were of the same serological type and that they differed serologically from influenza virus. Before the tests could be repeated on a larger scale, an event occurred which abruptly altered the character of the investigation. A change had just been made to a new source of supply of Swiss mice, when suddenly all the strains of virus appeared to undergo a remarkable increase in virulence, so that 80% of all mice inoculated died in a few days with extensive pulmonary consolidation. This was first interpreted as being an increase in virulence of the virus or perhaps an increased susceptibility on the part of the new mice. These explanations, however, proved not to be correct since a retest with sera which had formerly been protective showed that they no longer had any protective power. The conclusion, therefore, seemed justifiable that a new virus had been accidentally encountered.

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[3] Francis, T., Jr., and Magill, T. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 134.

The source of this virus was next investigated. Fifty normal mice of the variety in use were killed and autopsied; in 5 of these small areas of pulmonary consolidation were found, and in 12 others pin-point lesions were noted. An emulsion made from lungs showing these small lesions when passed by intranasal inoculation in a series of Swiss mice initiated a strain of the virus. Moreover, emulsions made from lungs appearing normal on gross inspection have also given rise to strains of this virus when passed from mouse to mouse by the intranasal route. The placing of apparently normal mice of the same strain in close cage contact with experimentally inoculated mice does not result in obvious pulmonary infection of the healthy contact mice. To date this virus has been recovered from normal Swiss mice obtained from 3 different sources of supply.

The characteristics of this virus disease are as follows: usually lung lesions are produced in the first series of mice to which the normal lung suspension is administered. Deaths begin to occur in the inoculated mice between the fourth and seventh passages, after which a high mortality rate is sustained. Death often takes place 48 hours after inoculation, occasionally 24 hours after. The areas of pulmonary consolidation are frequently smooth and grayish in appearance, but are often plum colored and similar in appearance to the lesions produced in the lungs of mice by human influenza virus. Microscopic sections show extensive cellular infiltration, chiefly mononuclear, and varying degrees of hemorrhage and edema. The virus is virulent in dilutions as high as 1:1,000,000; many mice inoculated with this dilution show well-developed pulmonary lesions, although some die without definite pulmonary consolidation. In general the mortality rate is considerably less with high dilutions of the material. The virus is readily filtrable through a Berkefeld V filter and collodion membrane with a pore diameter of 551.4 wov.

The serum of a rabbit given 3 intraperitoneal injections of mouse lung suspension containing virus did not appear to contain protective substances for mice against the virus. Weak and irregular protection was, however, obtained with certain normal human sera and with one anti-influenza virus serum.

Intranasal inoculation of ferrets produced a sharp early rise of temperature to about 105°, followed occasionally by a secondary rise. At times the ferrets showed respiratory symptoms. Some of the ferrets when retested later were found to be refractory to a second inoculation, suggesting that infection is followed by immunity. Mice, however, were not rendered immune by subcutaneous injections of the virus.

Mice are not susceptible to intracerebral, subcutaneous, or intraperitoneal injections of the virus. No effect is produced in rabbits by injection of virus either intraperitoneally or intracerebrally. Intratracheal instillation of the virus suspension in rabbits, however, resulted in an extensive pneumonia, mediastinitis and pericarditis. This infection was complicated by the presence of secondary bacteria. Guinea pigs were not affected by subcutaneous injection of the virus.

This short account indicates the presence in the lungs of certain strains of Swiss mice of a filtrable virus. This virus does not seem to cause obvious spontaneous disease, the carriers appearing healthy. There are present, however, in the lungs of a certain percentage of these mice easily visible macroscopic lesions. The virus seems to be present mainly in the respiratory tract and experimental infection takes place only after inoculation of the virus into the respiratory tract. Ferrets and perhaps rabbits are susceptible to infection with this virus. Subcutaneous and intraperitoneal inoculation of the virus does not result in infection in any of the species of animals used.

9358

Utilization of Vitamin B_1 From Fullers' Earth Adsorbates.

J. C. KERESZTESY AND W. L. SAMPSON. (Introduced by A. N. Richards.)

From Research Laboratories of Merck & Co., Inc., and Merck Institute of Therapeutic Research, Rahway, N. J.

We reported[1] our results with various methods of eluting vitamin B_1 from the international standard acid clay. The outcome of these experiments indicated that the vitamin on the acid clay was not entirely available to the test animal since the quinine sulfate extract was more effective than the clay itself in causing a remission of the symptoms of polyneuritis in rats. The experiments here reported were carried out to confirm those observations with a fuller's earth adsorbate of the vitamin.

One hundred cubic centimeters of a solution of crystalline vitamin B_1 containing 2500 curative doses, as determined by the Ammerman and Waterman[2] modification of the Smith curative procedure,

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[1] Sampson and Keresztesy, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 30.

[2] Ammerman and Waterman, *J. Nutrition*, 1935, **10**, 25.

TABLE I.

		Rat Assay			
Substance	Quantity	Dose in cc. or mg.	No. of rats	% cured	Calculated Total doses
1. Vit. B_1 solution	100 cc.	0.04 cc.	11	64	2500
2. Fullers' earth adsorbate	22 gm.	14.0 mg.	15	73	1570
3. Quinine extract of 2.	≎ 22 gm.	≎ 9.16 mg.	11	73	2400
4. Filtrate from F.E.	100 cc.	1.0 cc.	6	0	<100

were treated with 20 gm. of English fuller's earth. The recovered fuller's earth after careful drying weighed 22 gm. The filtrate, together with a portion of this fuller's earth, was set aside for direct feeding to the test animals. Another portion of the earth adsorbate was extracted with quinine sulfate after the method of Williams, *et al.*[3] Table I gives the results of our assay of these preparations.

These data show that the vitamin was completely removed by the fuller's earth since the spent filtrate from the adsorption was practically devoid of vitaminic activity. When a comparison of the original vitamin solution with the fuller's earth adsorbate prepared from it is made, it appears that some 900 doses were lost in the simple process of adsorption. However, the results obtained with the quinine sulfate extract of this fuller's earth indicate a complete recovery of the vitamin.

The fact that only about 60% of the vitamin can be accounted for when the activated fuller's earth is administered directly makes it apparent that the vitamin B_1 depleted rats cannot fully utilize the vitamin present in preparations such as fuller's earth adsorbates. This is in agreement with our results with the international standard.

9359 P

Hepatic Excretion in the Dog Following Oral Administration of Various Bile Acids.

HENRY DOUBILET.* (Introduced by L. Gross.)

From the Laboratories of the Mount Sinai Hospital, New York.

Cholic and desoxycholic acids when administered orally to man in large amounts increase both the volume of hepatic bile excretion as well as the concentration of the bile acids.[1] It was found that

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[3] Williams, Waterman and Keresztesy, *J. Am. Chem. Soc.*, 1934, **56**, 1187.

* Ralph Colp Fellow in Physiology.

[1] Doubilet, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 50.

TABLE I.
Average Bile Acid Excretion in Biliary Fistula Dogs after Oral Administration of Various Bile Acids.

gm.	Bile Acid Given	% Concentration			Gm. Bile Acid Excreted			Vol. of Bile cc.
		Cholic Acid	Desoxy-cholic Acid	Total Bile Acid	Cholic Acid	Desoxy-cholic Acid	Total Bile Acids	
	(Normal Excretion)	1.52	0.75	2.27	2.25	1.12	3.37	150
4	Cholic	1.82	1.31	3.13	4.46	3.21	7.67	245
4	Desoxycholic	0.34	1.39	1.73	0.95	3.91	4.86	281
8	Cholic	1.56	0.93	2.49	5.65	3.14	8.79	356
3	Desoxycholic	0.53	2.20	2.73	1.52	7.07	8.56	321
8	Glycocholic	1.90	1.43	3.33	5.23	3.76	8.99	282
6	Pure Ox Bile Salts	1.39	1.70	3.09	3.68	4.50	8.18	265
8	,, ,, ,, ,,	1.92	1.67	3.59	6.11	5.15	11.26	317
6	Dehydrocholic	0.22	0.71	0.93	1.12	3.62	4.74	510
8	,,	0.32	0.60	0.92	1.40	2.62	4.02	440
8	Dog Gall Bladder Bile Acids	2.51	1.93	4.44	5.63	4.29	9.92	230

cholic acid was much more effective in raising the concentration of the bile acids in hepatic bile. Similar experiments were carried out in the dog.

A common duct fistula, which drained all the hepatic bile sterilely into a rubber bag, was prepared in 3 female dogs. At various intervals the bags were emptied and the bile discarded after analysis. Pure cholic, desoxycholic, glycocholic and dehydrocholic acids, as well as pure ox bile salts were used. Various amounts of the bile acids were dissolved in equivalent amounts of alkali and administered to the dogs by stomach tube. The ox bile salt powder was mixed with milk for administration. To wash down the bile salt solution about 100 cc. of milk was used. In addition, collected canine gall bladder bile was analyzed for bile acids and an amount containing 8 gm. of bile acids was fed to the dogs. The dogs were kept on the ordinary animal room diet of oatmeal and meat scraps. The bile was analyzed for cholic acid, desoxycholic acid, total bile acids, taurine-conjugated bile acids, and glycine-conjugated bile acids by a method previously described.[2]

The average figures are summarized in Table I. The animals excreted 150 cc. of bile containing 3.37 gm. bile acids in a concentration of 2.27%. Feeding 4 gm. of cholic acid increased the concentration of both cholic and desoxycholic acid. When 4 gm. of desoxycholic acid were administered, the concentration of cholic acid fell, while that of desoxycholic acid rose. When the administration of these 2 bile acids was doubled in amount, each acid tended to depress the excretion of the other acid. The result was that the total excretion of bile acids was approximately the same, 8.79 gm. after cholic acid, and 8.56 gm. after desoxycholic acid.

When 8 gm. of glycocholic acid were administered, the concentration of bile acids rose to 3.33% and 8.99 gm. of bile acids were excreted. Glycocholic acid was found in the bile. On administering 8 gm. of pure ox bile salts (containing about 80% cholic and 20% desoxycholic acids) the concentration of bile acids rose to 3.59% and the total output to 11.26 gm. When only 6 gm. of ox bile salts were given, both the concentration and total output of bile acids were somewhat lower. Glycocholic acid appeared in the bile on ox bile salt administration. After the administration of 8 gm. dehydrocholic acid, the volume of bile rose to 440 cc. but the concentration of bile acids was only 0.92% while the total output was 4.02 gm. Six gm. of dehydrocholic acid gave approximately the same results.

The administration of 8 gm. of natural canine bile salts as found in normal gallbladder bile led to the excretion of a rather small volume of bile with a bile acid concentration of 4.44%. The total bile acid output was 9.92 gm.

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[2] Doubilet, H., *J. Biol. Chem.*, 1936, **114**, 289.

It has been shown by various investigators[3, 4] that the liver often responds to injury by the excretion of a very large volume of dilute bile. As the function of the liver improves, the bile becomes more concentrated and decreases in volume. When such an index of efficiency in the hepatic excretion of bile acids is used, *i. e.,* the largest excretion of bile acids in the smallest volume of bile, it would appear that the various bile acids used can be grouped in the following order of decreasing efficiency: canine bile acids, ox bile salts, glycocholic acid, cholic acid, desoxycholic acid, dehydrocholic acid.

Summary. Oral administration of large amounts of the various bile acids to dogs with a common duct fistula indicate that, from the point of view of largest excretion of bile acids in most concentrated form, the natural bile acids of the dog were probably the most efficient. Under the conditions of these experiments pure ox bile salts and glycocholic acid were found to be more efficient than the unconjugated cholic and desoxycholic acids. The natural bile acids (cholic and desoxycholic acids, and their various conjugated forms) were more effective than dehydrocholic acid.

9360

Hemolytic Streptococcus Toxins and Antitoxins. VI. A Strain of Hemolytic Streptococcus of High Toxicogenicity.

Leo Rane and Louise Wyman. (Introduced by Elliott S. Robinson.)

From the Antitoxin and Vaccine Laboratory, Massachusetts Department of Public Health, Jamaica Plain, Mass.

During an investigation dealing with the titration of hemolytic streptococcus toxins by the flocculation reaction,[1] one strain was found to produce unusually potent toxin. This strain was received from Dr. Alice C. Evans of the National Institute of Health, who designated it as "Streptococcus 594".* The interest of Doctor

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[3] Walters, W., Greene, C. H., and Fredrickson, C. H., *Ann. Surg.*, **91.**

[4] Bergareche, J., *Arch. de med., cir. y especialid*, 1933, **36**, 189.

[1] Rane, L., and Wyman, L., *J. Immunol.*, 1937, **32**, 321.

* "Streptococcus 594 was received from Dr. Krause of the Franz-Josefspital, Vienna, Austria. It was isolated from a case of scarlet fever." *Public Health Rep.*, 1934, **49**, 1385.

Evans in this organism was limited, however, to its phagological activity.

While the Dochez NY 5 strain of hemolytic streptococcus has long been considered one of high toxicogenicity, we have shown that the toxin as usually prepared is comparable to weak diphtheria toxins in requiring a long period of incubation to flocculate. Only after concentration does it flocculate as rapidly as strong diphtheria toxins. The toxin of strain 594, on the other hand, is of such potency that rapid flocculation is obtained directly without concentration.

Toxins of strain 594, with values ranging from 10 Lf to 80 Lf,† were prepared in a variety of infusion and infusion-free media.[1] In infusion-free media the brand and even the particular batch of peptone appreciably affects the Lf value. With this strain a toxin of higher potency is produced when purified maltose rather than dextrose or dextrose plus maltose is used as the source of carbohydrate, a fact which may be explainable in part by the slower utilization of maltose. The gradual addition of the sugar as a sterile 50% solution up to a concentration of 2% is advantageous. During the growth of the organism it is essential to neutralize the acid formed with sterile 5N NaOH added aseptically.

When the original culture was plated out, 2 types of colonies were apparent. One was smooth and glistening; the other, somewhat rough and dull. The latter appeared to produce toxin of higher potency than the former.

The toxin was lethal for chinchilla rabbits. Five cc. (180 Lf), given subcutaneously, killed rabbits weighing at least 5 pounds usually within 48 hours. With an increase in the Lf value, the M.L.D. was considerably lower. The rabbits could be protected by the use of antitoxin. Rabbits weighing about one pound were relatively immune and could tolerate many times the dose lethal for adult rabbits. Other breeds of rabbits were not as susceptible as the chinchilla.

Swiss mice, ordinary albino mice, chinchilla mice, guinea pigs and chicks were not affected by the concentrated toxin in doses of 1.0 cc. for the mice, 5.0 cc. for guinea pigs, 1.0 cc. for 7-8-day-old chicks, although 1.0 cc. was fatal to adult chinchilla rabbits.

The crude toxin combined and rapidly flocculated with antitoxin prepared in horses by the injection of the Dochez NY 5 toxin (Table I).

In horses the toxin has proved highly antigenic. With less than

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† 1 Lf as obtained by flocculation is equal to approximately 60,000 S.T.D. as determined by the intracutaneous rabbit test.

TABLE I.
Antitoxic Content of Horse Serums.

	Units Indicated by Flocculation of		
Horse No.	Concentrated NY 5 Toxin 28.8 Lf	Concentrated NY 5 Toxin 37.2 Lf	Unconcentrated 594 Toxin 36 Lf
623	230	232	225
629	240	232	232
633	144	146	144
638	160	162	156
666	113	113	112

50 cc. of toxin (36 Lf/cc.) two horses developed over 25 units of antitoxin within 3 weeks. This antitoxin readily neutralized NY 5 toxin both *in vivo* and *in vitro*. In all cases the same unit values were obtained with the NY 5 toxin and the homologous 594 toxin.

Summary. The highly toxicogenic property of a strain of hemolytic streptococcus is described. The general employment of this toxin for antitoxin production must await the results of additional research on the multivalency of the antitoxin.

9361 P

Sodium-d-Lactate Blood Clearance as a Test of Liver Function.*

LOUIS J. SOFFER, D. ALFRED DANTES AND HARRY SOBOTKA.

From the Laboratories and the Medical Service of Dr. George Baehr of the Mount Sinai Hospital, New York City.

We have described[1, 2] the metabolism of sodium-d-lactate in normal individuals and in patients with acute diffuse parenchymal disease of the liver. The results obtained suggested the possibility of the use of this substance as a test for liver function. It should be emphasized that the metabolism of sodium-d-lactate is quite different from that of the racemic or the l-salt with which all previous work has been done.

The test is performed before breakfast, and consists of the intravenous injection of 75 mg. per kilo of body weight of a 10 to 14%

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* Aided by a Grant from the Committee on Scientific Research of the American Medical Association.

[1] Soffer, L. J., Dantes, D. A., Newburger, R., and Sobotka, H., *Arch. Int. Med.* In press.

[2] Soffer, L. J., Dantes, D. A., and Sobotka, H., *Arch. Int. Med.* In press.

solution of sodium-d-lactate. A control sample of blood, collected in fluoride, is obtained just before the injection of the salt, and again 30 minutes after the injection, and blood lactic acid determinations are made.[3] All determinations are done in duplicate.

In normal individuals the blood lactic acid either falls below or remains very slightly above the control level at the end of 30 minutes, whereas in patients with hepatitis there occurs a distinct delay in the utilization of the injected d-lactate. We consider an elevation of 5 mg. % or more above the control blood lactic acid level at the end of ½ hour as evidence of liver damage.

The advantage of this test lies in the fact that it may be used in the presence of jaundice and thus serve in the differentiation between extrahepatic obstructive and non-obstructive icterus.

TABLE I.
Deviation of Blood Lactic Acid from Control Level 30 Minutes after Injection.

No. of Cases	Diagnosis	Deviation in mg. %		
12	Normal	—7.3	—1.1	+1.4
		—5.5	—0.8	+2.1
		—4.4	—0.2	+3.0
		—1.3	+0.5	+3.2
6	Catarrhal Jaundice	+8.3	+9.9	+12.2
		+9.4	+10.8	+17.6
4	Arsphenamine Jaundice	+6.3	+9.6	
		+7.8	+26.1	
2	Common-Duct Obstruction Carcinoma of Head of Pancreas	—5.5	—4.4	
1	Common-Duct Stone	—1.3		
3	Diabetes Mellitus	—3.5	—0.8	+4.4
1	Myotonia Congenita	—1.1		
1	Myasthenia Gravis	—0.8		

Our limited investigations at present suggest that the results obtained with this test are not influenced by either the coincidental presence of diabetes mellitus or muscular disease. The conversion of the injected lactate is entirely dependent on the integrity of the liver.

No undue reactions were observed in any of our patients.

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[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

9362

Absorption of Iron Compounds from the Upper Part of the Small Intestine.

Juda Groen* and F. H. L. Taylor. (Introduced by G. R. Minot.)
From the Thorndike Memorial Laboratory, the Boston City Hospital, and the Harvard Medical School, Boston, Mass.

The intubation technique of Miller and Abbott[1, 2, 3] as modified by Groen[4] has been used in the present investigation to study the absorption of ferrous, ferric, and complex iron compounds from a 50 cm. loop of the small intestine in man.

Each iron salt after being dissolved in 100 ml. of distilled water was introduced into the isolated segment of gut of a fasting subject in such amounts that between 80 and 100 mg. of iron were present.

After 30 minutes the solution was aspirated and washing of both the blocked segment of intestine and the stomach was commenced and was continued for 30 minutes.

Aliquot amounts of both intestinal contents and washings were analyzed for total iron by the method of Taylor and Brock.[6] When the intestinal contents contained organic debris of a nature that made accurate sampling impossible, the intestinal contents were partially digested by standing for 48 hours after mixing with an equal volume of concentrated sulphuric acid.

With ferric salts (ferric ammonium sulphate, ferric chloride and ferric nitrate) 20 to 35% of the iron was recovered. With the ferrous salts (ferrous sulphate, ferrous ammonium sulphate and ferrous chloride) 60 to 76% of the iron administered was recovered. With ferric ammonium citrate 100% was recovered. Dr. Wilfrid Owles[11] in this laboratory, using a 3-lumen tube and a completely isolated segment of intestine, obtained similar recoveries for ferrous sulphate and ferric ammonium citrate, while Abbott and Miller reported[3] 100% recovery with ferric ammonium citrate. If the difference between the amount of iron ingested and that recovered, were assumed to be the amount absorbed, the conclusion would be that ferric salts were better absorbed than ferrous salts and that ferric ammonium citrate was not absorbed at all. This conclusion is obviously at variance with clinical observations on patients suffering from achlorhydria and anemia due to iron deficiency.[7–10]

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* Rockefeller Foundation Fellow (1935-1936).

[1] Miller, T. G., and Abbott, W. O., *Am. J. Med. Sci.*, 1934, **187**, 595.

[2] Miller, T. G., and Abbott, W. O., *Ann. Int. Med.*, 1934, **8**, 85.

[3] Miller, T. G., and Abbott, W. O., *J. A. M. A.*, 1936, **106**, 16.

[4] Groen, Juda, *J. Clin. Invest.*, 1937, **16**, 245.

[5] Brock, J. F., and Taylor, F. H. L., *Biochem. J.*, 1934, **28**, 447.

[6] Taylor, F. H. L., and Brock, J. F., *Biochem. J.*, 1934, **28**, 442.

[11] Owles, W. H., personal communication.

The possibility that the discrepancy between the amount of iron administered and that recovered might be explained by adsorption of the iron compound on the mucous lining of the intestine was checked by a second series of observations. The technique employed was the same except that following the 30-minute period allowed for absorption washing was continued for periods up to 3½ hours. The washings for each half hour were collected and separately analyzed for iron. It was found that the intestine slowly yielded up additional amounts of iron. These additional amounts of iron coincided with the appearance in the washings of particles and filaments of rusty brown colored mucus, which were rich in iron. When the washings were continued for 3½ hours 100% of all forms of iron were recovered. It appeared that the highly ionizable iron salts entered into combination or were adsorbed by the mucus, while ferric ammonium citrate possessing little "astringent" properties was not.

Heath, *et al.*,[12] have shown that, when iron is given with mucin to patients with hypochromic anemia, there is inhibition of the response of the patient's blood. We thus mixed iron salts with commercial mucin prior to administration so that interaction with the protein could take place. When this was done the recovery of iron was quantitative after 30 minutes of washing. These experiments would suggest that the apparent absorption obtained by Owles and ourselves and also by Miller and Abbott[3] was actually due to adsorption on the mucus of the intestinal tract.

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[7] Heath, C. W., Strauss, M. B., and Castle, W. B., *J. Clin. Invest.*, 1932, **11**, 1293.

[8] Witts, L. J., and Manch, M. D., *Lancet*, 1936, **1**, 1.

[9] Witts, L. J., and Davidson, L. S. P., *Proc. Roy. Soc. Med.*, 1933, **76**, 607.

[10] Davidson, L. S. P., Fullerton, H. W., and Campbell, R. M., *Brit. Med. J.*, 1935, **11**, 195.

[12] Heath, C. W., Alsted, G., Pohle, F. J., and Minot, G. R., personal communication.

9363 P

Effect of a Heat-Resistant Enzyme Upon the Antigenicity of Pneumococci.

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From the Hospital of the Rockefeller Institute, New York.

Rabbits immunized by the intracutaneous injection of heat-killed encapsulated pneumococci fail to develop the type-specific carbohydrate antibodies which result from the intravenous injection of the same antigen.[1] An attempt was made to follow the fate of pneumococci injected into the skin, in the hope of determining the cause of this lack of type-specific antigenic response.

Heat-killed cells of pneumococcus Type I were injected at several sites into the skin of a rabbit. The injected areas were excised at different intervals of time and films made from the tissue fragments were stained by the Gram technic. There was, of course, a pronounced polymorphonuclear infiltration at the site of injection. The pneumococci were seen to undergo a process of extracellular digestion which began within 24 hours after injection and was completed in 4 to 5 days; many bacteria became Gram negative before being engulfed by the leucocytes. These observations suggested that leucocytes produce ferments capable of attacking heat-killed pneumococci.

To demonstrate the existence of these enzymes, a polymorphonuclear exudate was obtained by injecting aleuronate into the pleural cavity of a rabbit. The washed cells extracted with N/10 HCl yielded a soluble principle which has the property of rendering pneumococci Gram negative. Active extracts, with similar properties, were obtained from the organs—especially the liver, pancreas, spleen, and lungs—of several animal species. The same enzyme was also prepared from the pleural exudate from a tuberculous patient, who developed empyema following secondary infection with *H. influenzæ.*

The active enzyme present in these extracts is heat-resistant, especially at slightly acid reactions. Its rate of activity upon pneumococci increases with temperature up to 75°C.; the range of pH activity lies between pH 5.5 and pH 9.5. The enzyme appears to be a protein which is rapidly destroyed by pepsin but is completely resistant to trypsin and chymotrypsin.

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[1] Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441.

As stated above, the purified enzymic preparation renders heat-killed pneumococci Gram negative, whether the bacteria are R or S variants, and irrespective of type-derivation. The cells, however, do not undergo dissolution but retain their characteristic morphology; the turbidity of the bacterial suspension is also very little altered.

Following the repeated intravenous injection of heat-killed pneumococci Type I which have been digested with the enzyme, no precipitins for the capsular polysaccharide appear in the serum, while these invariably occur if the untreated cells are injected. It appears, therefore, that the enzyme has the property of inactivating the capsular antigen of virulent pneumococci.

The enzyme, however, does not decompose the capsular polysaccharide itself. In fact of all the soluble substrates tested, yeast nucleic acid* was the only one to be attacked by the purified preparations. A description of this reaction is presented elsewhere.[2]

It is interesting to point out that some preparations of crystalline trypsin† and chymotrypsin† were found to exhibit a small measure of activity against the Gram positive structure of pneumococci and against yeast nucleic acid. After repeated recrystallizations, however, the proteolytic enzymes no longer have any action upon either of these substrates.

9364

Differentiation of Blood Groups in Dogs Based on Antigenic Complexes Present in the Erythrocytes.*

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From the Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven.

When dogs are subjected to prolonged, intensive plasmapheresis, the hematocrit values tend to fall to anemia levels in spite of the

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* The authors are indebted to Dr. P. A. Levene for a sample of yeast nucleic acid.

[2] Dubos, R., *Science*, 1937. In press.

† The authors are indebted to Doctors J. H. Northrop and Dr. M. Kunitz for supplying several samples of crystalline trypsin and chymotrypsin.

* The expenses of this investigation were defrayed by a grant from the Research Fund, Yale University School of Medicine, and by an Alexander Brown Coxe Fellowship Grant.

† Alexander Brown Coxe Fellow, 1936-37.

reinjection of the cells removed in the bleedings of the previous days. To compensate for this destruction of erythrocytes *in vivo,* it is necessary to inject periodically red blood cells obtained from donor animals. Although dogs are generally compatible with each other as regards blood transfusions[1] and this is evidenced by successful initial injections of cells, subsequent transfusions may create a sensitization which renders the donors incompatible thereafter.[2–5] The development of isoagglutinins and isohemolysins results in the production of an intense hemoglobinuria; the continuation of the cell infusions, spaced from 7 to 10 days apart, is followed by symptoms of profound shock.

It was suggested[3] that the development of this incompatibility is due to a common mechanism—a sensitization induced in the recipient by repeated injections of an antigen in the red blood cells of the donor. The evidence for this belief may be summarized as follows:

(1) The clinical picture of the reaction obtained in our dogs is characteristic of anaphylactic shock in that species.

(2) With the lapse of time (5 to 10 weeks) there is a restoration of compatibility. However, in this condition the sensitiveness may easily be made manifest again within one week by injection of the antigen.

(3) The incompatibility is "one-sided" in that the sera of the recipients agglutinate and hemolyze the cells of the donors, whereas cells of the recipients are compatible with the sera of the donors.

(4) The incompatibility can be passively transferred to a normal non-sensitized dog.

(5) Finally, the sensitized animals when in shock respond immediately and favorably to the intracardiac administration of adrenalin.

When the sera of the sensitized dogs were subjected to microagglutination tests *in vitro,* employing the cells of 36 dogs chosen at random, we noted that approximately 50% of these animals were incompatible with the sensitized dogs although none of the entire group had ever served as donors. This incompatibility, which proved to be independent of breed or sex in our series of animals, is probably due to these particular dogs having an antigenic make-up in their red blood cells similar to that of the original donors. To facilitate discussion the symbol, α, has been assigned to that group still compatible with the sensitized dogs; the symbol, β, to the incompatible group.

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[1] Wiener, A. S., *Blood Groups and Blood Transfusion,* Springfield, Ill., Charles C. Thomas, 1935.

[2] Ottenberg, R., Kaliski, D. J., and Friedman, S. S., *J. Med. Res.,* 1913, **28**, 141.

[3] Melnick, D., Burack, E., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 616.

[4] Wright, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 440.

[5] Melnick, D., and Cowgill, G. R., *Am. J. Physiol.,* 1937, **119**, 70.

Five dogs of the compatible group, α, were subsequently employed as blood donors, enabling us to continue our studies of serum protein regeneration. Two of the sensitized dogs (α dogs) have received to date from these donors a total of 38 infusions of red blood cells, spaced for the most part 7-14 days apart. No evidence of an incompatibility subsequent to these injections has been observed, nor has the appearance of any agglutinins for the cells from these donors been noted. The non-development of antibodies for these cells after the numerous infusions indicates that the present donors will remain compatible with the sensitized dogs. Apparently these donor animals have an antigenic grouping in their red blood cells similar to that of the recipients.

We are now in a position to report further observations on blood grouping in dogs by carrying out experiments which are essentially the reverse of those described above. These were made in the course of our present investigations of serum protein regeneration.

Dog No. 3, described in a previous communication[3] and a member of the α group, received 150 cc. of cells obtained from an animal previously demonstrated to be a member of the incompatible (β) group. Within the following week marked agglutination *in vitro* was noted when cell suspensions of this donor were added to the sera of the recipient. This sensitized animal has been used as an indicator to enable us to group satisfactorily all dogs used in our subsequent studies.

Three dogs of the β group, this time acting as recipients, have received 16 injections of cells from 4 donors of the β group without developing any signs of an incompatibility. One of these animals actually received a total of 11 cell infusions. Micro-agglutination tests also indicated the complete absence from the sera of these recipients of agglutinins for cells of the β group. Apparently all of these dogs, members of the β group, possess the same antigenic complex in their red blood cells. These results are analogous to those obtained when both recipient and donor were of the α group.

Four dogs of the β group were subjected to 35 cell injections from 5 animals of the α group with no untoward effects being manifest. The sera of the recipients were also observed to contain no agglutinins or hemolysins for the cells of the donors. These findings are in direct contrast to those noted when conditions were reversed, namely, when the recipients were members of the α group and the donors of the β group. Apparently the erythrocytes of dogs of the β group possess all of the antigenic groupings present in the cells of animals of the α group and in addition contain some other complex which may act as a foreign body when injected into dogs only of the α group. Therefore, if it is true that 50% of the dogs belong to either the α or β group and if these are the only 2 groups present in that species, it follows that there is only one chance out of 4 that an incompatibility will occur when a dog is subjected to repeated infusions of red blood cells from a donor animal. This is obvious when reference is made to the table which summarizes our findings. It may be for this reason that in Whipple's laboratory,[4] where animals were subjected to repeated injections of erythrocytes from a group of donor animals, hemoglobinuria and shock were observed with some of the dogs whereas others received similar exchanges of red cells from the same group of donors without untoward effects.

TABLE I.
The Results of Repeated Injections of Red Blood Cells into Dogs.

Blood Group of		
Donors	Recipients	Results
β	α	Blood incompatibilities develop in recipients
α	α	Dogs remain compatible.
β	β	,, ,, ,,
α	β	,, ,, ,,

9365 P

Respiratory Effects of Substituted Phenols at Varying Carbon Dioxide Tensions.

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During a study of the reversible inhibition of cell division which is produced in fertilized marine eggs by certain nitro and halophenols[1, 2] the CO_2 tension was found to be a significant factor in the degree of inhibition attainable.[3] The respiratory effects of substituted phenols were then measured on cells which were exposed to a gas phase containing oxygen and CO_2 in various ratios.

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[1] Clowes, G. H. A., and Krahl, M. E., *Science*, 1934, **80**, 384.

[2] Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, 1936, **20**, 145.

[3] Krahl, M. E., Clowes, G. H. A., and Taylor, J. F., *Biol. Bull.*, 1936, **71**, 400.

The manometric techniques of Warburg (direct method) and of Dickens and Simer (second method) were employed.[4] Fertilized eggs of the sea urchin *(Arbacia punctulata)* and a baker's yeast (Anheuser-Busch) served as material. The egg experiments were made at 20°C., using sea water as the suspension medium. This was 0.002 molar in bicarbonate. The yeast experiments were made at 25°C., using a suspension medium 0.1 molar in glucose and 0.004 molar in bicarbonate.

When the oxygen consumption of either type of cell is plotted against the logarithm of the concentration of a stimulating substituted phenol, the curve rises nearly linearly until an optimum is reached; beyond this optimum concentration the oxygen consumption falls rapidly, nearly to zero. Cell division in the fertilized eggs is inhibited by those concentrations of the reagent which give the falling portion of the oxygen consumption curve.[1, 5]

With fertilized sea urchin eggs in 4,6-dinitro-o-cresol a change in the partial pressure of CO_2 from the usual atmospheric level, about 0.0005 atmospheres, over a range of tensions up to 0.02 atmospheres, has little effect on the position of the rising portion of the oxygen consumption curve. The falling portion of the oxygen consumption curve is displaced to a lower range of concentrations of the 4,6-dinitro-o-cresol; the respiratory optimum concentration decreases from 8×10^{-6} to 10^{-6} molar; and the number of units of excess oxygen consumed at this optimum decreases with rising CO_2 partial pressure. In 0.02 atmospheres CO_2 the oxygen consumption of the eggs, without the substituted phenol, is 0.68 cmm. per hour per mg. eggs (dry weight) as compared with the value of 1.7 in the absence of CO_2. Hence, while the rate of oxygen consumption of eggs in normal sea water is sensitive to increase in CO_2 partial pressure, the oxygen consumption of eggs in concentrations up to 10^{-6} molar 4,6-dinitro-o-cresol is, in great measure, independent of the changes in CO_2 partial pressures up to 0.02 atmospheres.

With yeast in 4,6-dinitro-o-cresol, increase in CO_2 partial pressure from 0.05 to 0.15 atmospheres causes little or no shift in the rising portion of the oxygen consumption curve, a marked displacement of the falling portion to lower concentrations, and a decrease in the optimum value of Qo_2.

With yeast in 2,4-dichlorophenol, increase in CO_2 partial pressure from 0.05 to 0.15 atmospheres causes a considerable shift of the rising portion of the oxygen consumption curve to a higher range of concentrations and only a very small shift in the falling portion of the curve.

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[4] Dixon, M., *Manometric Methods,* Cambridge University Press, 1934.

[5] Krahl, M. E., and Clowes, G. H. A., *J. Gen. Physiol.,* 1936, **20**, 173.

Increasing CO_2 tension produces a tendency toward increase in intracellular hydrogen ion concentration.[6] The pH effects dealt with here are primarily on the cell interior and therefore to be distinguished from those obtained by Field,[7] who was chiefly concerned with changes in extracellular pH.

At the ionic strengths used here 4,6-dinitro-o-cresol has a pK′ of about 4.4;[8] 2,4-dichlorophenol a pK′ of about 7.7.[9] Thus at the normal intracellular cytoplasmic pH of about 6.5 to 7.0, 4,6-dinitro-o-cresol is almost completely in the dissociated form, while 2,4-dichlorophenol is chiefly in the undissociated form. In the first case, rising intracellular acidity produces a small relative change in the concentration of phenol anion and a large relative change in the concentration of undissociated phenol. In the latter case the situation is reversed. The probable effects of increased CO_2 tension on intracellular ionization of these substituted phenols in an interior aqueous phase thus appear to parallel the effects of CO_2 on the oxidative stimulating properties of these substances.

2,4,5-trichlorophenol occupies a middle position between 2,4-dichlorophenol and 4,6-dinitro-o-cresol in respect to the influence of CO_2 on its respiratory effect. The pK′ of 2,4,5-trichlorophenol is also intermediate between those of 2,4-dichlorophenol and 4,6-dinitro-o-cresol, being about 6.9.[8]

These experiments appear to provide some indication that oxidative stimulation may be favored by high intracellular concentrations of the dissociated form, while inhibition of oxidation and reversible block to cell division may be favored by high intracellular concentrations of the undissociated form of the substituted phenols.

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[6] Jacobs, M. H., p. 97 in Cowdry, E. V., *General Cytology*, Chicago, University of Chicago Press, 1924.

[7] Field, J., 2nd., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1342.

[8] Krahl, M. E., and Clowes, G. H. A., unpublished experiments. See also Field, reference 7, for a slightly higher value for 4,6-dinitro-o-cresol.

[9] *International Critical Tables*, New York, McGraw-Hill, 1929, Vol. VI, p. 271.

9366

Effect of Adrenotropic Extract upon the Accessory Reproductive Organs of Castrated Rats.

Charles S. Davidson. (Introduced by J. B. Collip.)
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A preliminary report[1] has been published concerning the effect of adrenotropic extracts of the pituitary upon the secondary sexual characteristics of castrate young male rats. Additional experiments confirming and enlarging upon this work are herein reported.

Enlargement of the prostate and seminal vesicles of young male castrates, associated with adrenal enlargement produced by treatment with adrenotropic extract, has been noted in this laboratory. The interpretation that the enlargement of the prostate and seminal vesicles depends upon the adrenal stimulation is supported by the additional experiments reported here.

1. *Effect of Adrenotropic Extracts upon Castrate Males.* Twenty-three-day-old male rats were castrated and allowed to rest for 5 days. On the sixth day injections of adrenotropic extract were begun and continued for 9 days, approximately 50 units being administered daily. The following day the animals were killed, the adrenals were weighed, and the prostates and seminal vesicles were weighed together. Tissues were fixed immediately and histological examination subsequently made.

Twelve animals, castrated and killed 15 days later, served as controls. The adrenal weights averaged 17 mg. (13-19 mg.) and the weights of the prostate and seminal vesicles averaged 39 mg. (28-47 mg.). Normal animals of the same age have adrenals of about 16 mg. and prostate and seminal vesicles averaging 100 mg. (82-128 mg.). In the experimental group the adrenal weights averaged 53 mg. (40-61 mg.) and the prostate and seminal vesicle weights averaged 56 mg. (50-63 mg.).

The enlargement of the prostate and seminal vesicles is definite. Histological examination showed marked repair of the prostate from the castrate level. The seminal vesicles showed little histological change, but were definitely larger than in the controls.

2. *Effect of Adrenotropic Extracts upon Hypophysectomized Castrate Males.* It has been known for some time that many nonspecific substances (foreign proteins, drugs, etc.) produce an en-

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[1] Davidson, C. S., and Moon, H. D., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 281.

largement of the adrenals. However, it is quite clear that only pituitary adrenal stimulating extracts are effective in the hypophysectomized animal.[2] It was felt necessary, therefore, to attempt to produce the secondary sexual changes in hypophysectomized castrated rats, to eliminate any non-specific effect of the extract.

Eighteen males were castrated at 30 days of age. Four days later they were hypophysectomized. Seven were then reserved as controls and the remaining 11 injected twice a day for 14 days with approximately 25 units of adrenotropic extract. On the 15th day (48 days old) the animals were killed, organs weighed and fixed. Hypophysectomy was complete in all cases.

The results summarized in Table I show a marked increase in weight of the prostate and seminal vesicles. Histological evidence again showed the typical changes in the prostate, much less so in the seminal vesicles.

TABLE I.

Adrenal Weights mg.		Prostate and Seminal Vesicle Weights mg.	
Experimental	Controls	Experimental	Controls
81	9	85	46
69	9.5	104	39
51	9	60	38
64	10	67	41
71	13	73	40
83	9	87	37
83	8	94	37
86		84	
57		84	
61		64	
56		65	
Aver. 67	9.5	72	40

The enlargement of the adrenals is to be noticed. Normal adrenal weights, in our colony, for animals 48 days old are between 25 and 30 mg. The enlargement here in the hypophysectomized animals is, therefore, to more than twice the normal weight. The enlargement is confined to the cortex, except that the vacuoles sometimes seen in the adrenal medulla of the hypophysectomized rat are absent. In fact, the medulla presents, on the whole, a much more normal appearance than the control. The cortical enlargement appears to be confined chiefly to cellular enlargement; no evidence of hyperplasia is to be seen.

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[2] Collip, J. B., Anderson, E. M., and Thomson, D. L., *Lancet*, 1933, Aug. 12, p. 347.

Half the animals were injected subcutaneously and the others intraperitoneally. No differences in effectiveness of the extracts administered by these 2 routes were noted.

3. *Effect of Adrenotropic Extracts upon Castrated Adrenalectomized Males.* To show that the effect of the adrenotropic extracts is through the adrenals and not a direct effect of the extract on the prostate and seminal vesicles, a series of adrenalectomized animals were treated with the extract. Fifteen 23-day-old males were castrated and adrenalectomized 2 to 5 days later. Eight were then injected for from 5 to 9 days with adrenotropic extract. Organ weights are shown in Table II. These results show that the effect of adrenotropic extracts on the secondary sex organs is through the adrenals, and does not occur in their absence.

TABLE II.

Prostate and Seminal Vesicle Weights mg.	No. of days treated
32	5
37	5
43	6
40	6
32	9
36	9
43	9
23	9
Aver. 37	

Controls averaged 40 mg. (35-45 mg.).

Summary. 1. The effect of adrenotropic extracts of the pituitary upon the prostate and seminal vesicles of castrate young rats, previously reported, has been confirmed. 2. The response may be elicited in hypophysectomized animals. 3. The adrenotropic extract is without effect in adrenalectomized castrated rats. 4. The adrenotropic extract used causes marked enlargement of the adrenal cortex in normal animals, and not only prevents the adrenal atrophy following hypophysectomy but enlarges the cortex to more than twice the normal size. Some changes in the medulla of the adrenal are also reported.

9367 P

Substitution of β-Alanine, Nicotinic Acid, and Pimelic Acid for Meat Extract in Growth of Diphtheria Bacillus.

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In the course of studies extending over several years, the attempt has been made to define chemically the requirements of certain strains of diphtheria bacilli, which would permit growth equal or superior to that occurring on the usual peptone-infusion broth. This has been accomplished, as regards the peptone portion, for some of the strains, by means of amino acids[1] and certain differences and similarities between strains have been brought out.

An investigation of the extractive portion of the medium, absolutely essential for growth with all our strains, has led to the recognition that at least 3 substances are required by 2 strains. These are β-alanine, nicotinic acid, and pimelic acid. For a third strain the pimelic acid can be omitted. Omission of either β-alanine or nicotinic acid reduces the amount of growth practically to nil in the case of all 3 strains.

The quantities required to produce maximal effect are of the order of one microgram per cc. for β-alanine and nicotinic acid, and about 0.025 microgram per cc. for pimelic acid.

Pimelic acid apparently has not before been encountered in biological systems. The presence in liver-extracts of an ether-soluble acid-substance, from which a methyl ester could be prepared and distilled either *in vacuo* or at atmospheric pressure was first established. A better source of material having the same growth-stimulating and chemical properties was found in cows' urine. From 100 gallons of this material 0.6 g. of crystals proving to be pimelic acid were obtained. Details of this work are now in press.[2]

From a liver-extract concentrate representing 300 kg. of liver, about 10 mg. of crystals showing the desired growth-promoting property were obtained, and identified as nicotinic acid. Identification was facilitated by knowledge of Knight's[3] recent findings in connection with the effect of this substance on growth of the staphylococcus.

The third factor, β-alanine, has not actually been isolated from liver-extract. The fraction containing the activity appeared to possess both COOH and NH_2 groups in equivalent proportions. Since a complete casein-hydrolysate was used in the control medium, β-alanine suggested itself as a possible component of the mixture and trial showed it to have practically the full effect of the liver-extract fraction. This compound, also, has previously been shown by Williams[4] to be of importance in the growth of certain yeasts, functioning as a part of the "bios" complex.

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[1] Mueller, J. H., *J. Bact.*, 1935, **29**, 515; Mueller, J. H., and Kapnick, I., *J. Bact.*, 1935, **30**, 525.

[2] Mueller, J. H., *J. Bact.* and *J. Biol. Chem.* In press.

[3] Knight, B. C. J. G., *Nature*, 1937, **139**, 628.

It is probable that other substances present in tissue-extract will increase somewhat the amount of growth of our test organisms. Thus, with the use of a complete tissue-extract, from 3.5 to 4.0 mg. of bacterial nitrogen are produced on 10 cc. of medium in 3 days' growth. On the same control base with the above 3 substances replacing the extractives, from 2.2 to 2.9 mg. of nitrogen are obtained. The usual peptone-infusion broth produces from 1.0 to 2.0 mg.

The following type-experiment illustrates the relative effect of the 3 substances on 3 strains of *C. diphtheriæ*—the National Institute of Health and the Alabama State Health Department's strains of the Park-Williams No. 8, and the "Allen" strain, isolated locally 2 years ago.

The relative amounts of growth are estimated by Arnolding 10 minutes, centrifuging and washing the organisms, and determining

TABLE I.

Composition of Medium		Mg. Bacterial Nitrogen N.I.H. Park 8	Ala. Park 8	"Allen" strain
1. Control* + ———		.07	.06	.08
2. " + pimelic acid 1γ (1)		(lost, very low)	.04	.10
3. " + nicotinic acid 10γ (2)		.02	.04	.21
4. " + β-alanine 10γ (3)		.10	.06	.08
5. " + (1) + (2)	amounts as above	.08	.00	.25
6. " + (1) + (3)		.08	.07	.10
7. " + (2) + (3)		.66	1.99	.64
8. " + (1) + (2) + (3)		2.32	1.86	2.24

*The control had the following composition:

Casein—HCl hydrolysate	.10 gm.
Cystine	.001 "
Glutamic acid	.050 "
Lactic acid (as Na salt)	.1 cc.
Salt mixture: NaCl	.050 gm.
$Na_2HPO_2 . 2H_2O$	.025 "
KH_2PO_4	.0035 "
$MgCl_2 . 6H_2O$	.003 "

In addition, the controls for the Park 8 strain contained 1.0 mg. tryptophane and 0.05 cc. ethyl alcohol. Quantities given are for 10 cc. of medium. The pH is adjusted to 7.4-7.6, the tubes autoclaved at 10 lbs. for 10 minutes, inoculated with a small loopful of pellicle from an 18 to 24-hour broth culture and incubated in a slanted position at 34°C. for about 60 hours.

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[4] Williams, R. J., and Rohrman, E., *J. Am. Chem. Soc.*, 1936, **58**, 695.

the nitrogen of the sediment by microkjeldahl as previously described.[5]

Increasing slightly the concentrations of nicotinic acid and β-alanine improves the growth somewhat, but one or more additional substances evidently must be supplied for maximal growth.

Conclusions. Minute amounts of nicotinic acid, β-alanine, and pimelic acid, present together, permit about two-thirds the maximal growth of the diphtheria bacillus obtained from whole tissue extracts when added to a suitable control medium. The pimelic acid appears to be the least essential of the three. Differences appear between strains, even of the Park 8 organism, which may well be multiplied when further strains are examined.

9368

The Antigonadotropic Factor. Origin and Preparation.

BERNHARD ZONDEK AND FELIX SULMAN.

From the Laboratory of the Obstetrical-Gynecological Department, Rothschild-Hadassah Hospital, Jerusalem.

One of us[1] pointed out the fact that in rodents the gonadotropic effect diminishes if prolan from human pregnancy urine is applied in a protracted manner. Since that time the phenomenon has been repeatedly studied. Collip, Selye, *et al.*,[2] explained this as being due to the effect of antihormones, and succeeded in proving in the test-tube experiment the antisubstances against the hormones.

Proof of the presence of an antigonadotropic factor can be furnished only by the impairment of the gonadotropic reaction in rodents. As shown by our experiments[3] proof of the antigonadotropic factor by serological methods is not obtainable, pure prolan not forming any precipitins or complement-fixing antibodies. The sera of experimental animals which had been "immunized" with pure prolan over a period of more than 2 months do not show any serological reaction; they evince, however, a highly antigonadotropic effect.

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[5] Mueller, J. H., *J. Bact.*, 1935, **29**, 383.

[1] Zondek, B., *Hormone d. Ovariums u. d. Hypophysenvorderlappens*, Berlin and Vienna, J. Springer, 1st ed., 1931, p. 159; 2nd ed., 1935, p. 272.

[2] Collip, J. B., Selye, H., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487, 566, 1113; 1935, **32**, 544.

[3] Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

The titration of our antigonadotropic sera was performed exclusively by impairing the production of the anterior pituitary reactions (HVR) in rodents. 0.5 cc. of an antigonadotropic serum or the corresponding amount of an antigonadotropic dry powder solution (see below) was mixed with prolan (the latter in increasing amounts), filled up to a total volume of 4 cc., placed in the incubator for 2 hours and finally injected into the experimental animals, infantile female rats 3-4 weeks old, weighing 25 to 30 gm. They were injected 4 times (if there were more than 4 cc. of the injection fluid, 6 times) at equal intervals (12 hours), spread over 36 hours. Sixty hours after the first injection we began with the vaginal smears, at intervals of 12 hours. One hundred and twenty hours after the first injection the animals were killed and ovaries and uteri examined.

One prolan rat unit is known to be the smallest amount of prolan able to produce the anterior pituitary reactions (HVR) I, II or III, in infantile female rats (Zondek[1]). We define as one prolan anti-unit (PAU) the smallest amount of the antigonadotropic factor able to prevent the anterior pituitary reactions (HVR) I, II or III, according to the above experimental order.

For the quantitative titration of the prolan the anterior pituitary reaction (HVR) I, *i. e.*, the bringing about of oestrus, is especially fitted. We hold that this method for the quantitative determination of the units is much more exact than the record of the mere increase in weight of the ovary. According to our experiments the weight increase is not in proportion to the prolan units and is not such a precise indicator as the oestrus reaction (HVR I). We applied as a minimum per rat 10 RU of prolan, which, in our control-experiments, always produced HVR I, II or III; but if the substance was added to an effective antigonadotropic serum these reactions were not obtained in any case.*

In a few cases we performed the titration of the antigonadotropic serum in mice and rabbits as well. These animals, however, cannot be compared with the rat for exactitude of the reaction, the latter being an especially sensitive and exact indicator for prolan A.

The antigonadotropic factor has its origin in the blood and is found especially in the serum. We can obtain it by repeated treatment of the experimental animals, the application of high doses of hormone does not seem to be as important as the daily repetition of the treatment with gonadotropic hormone. For preparation of the antigonadotropic factor we usually used the blood of rabbits, less frequently that of rats. In the rat we could observe the formation of the antigonadotropic factor following a prolonged application (twice a week subcutaneously), however the titre did not become very high. If we treated rats in this manner for one year twice weekly with 100 RU of prolan respectively after close of the treatment we still could observe swollen follicles and fresh corpora lutea, which is not obtainable, as is well-known, by daily treatment with gonadotropic hormone (Evans, Zondek).

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* The rat test can be performed proceeding with simultaneous or subsequent single or repeated applications of prolan and antiprolan. (Details will be given in a later report.) Antiprolan may be given a long time before or even after prolan, but, from reasons of principle, we kept to the 2 hours-incubator-contact-method *in vitro*.

Rabbits which were injected with 250 RU prolan 4 times a week subcutaneously and twice a week intravenously showed 2 months later underweight, atrophic, diffusely luteinized ovaries. The curve of the blood titre was approximately as shown in Table I.

TABLE I.

Duration of treatment weeks	Titre per cc. of serum PAU
4	10†
6	20
8	80
12	100
20	120
26	150

† 1 PAU = 1 prolan anti-unit (see above).

N.B. At least 10 units must be assayed in a test rat.

It is necessary to have an exactly titrated antigonadotropic factor always in stock and to be in a position to preserve and concentrate it. For that reason we worked out the following method: The serum to be examined is poured into 4 times its volume of acetone, whereat a finely flaked precipitation forms. The precipitate is instantly freed from the supernatant fluid on a Buchner or Seitz filter; after that it is washed 3 times with acetone, while being constantly stirred and withdrawn by suction, and finally washed again 3 times with ether. For 5 minutes air is drawn through the filter, until the precipitate is absolutely dry. The resulting fine rose-colored powder is preserved under vacuum in the exsiccator, and there it is preservable at least for 6 months. The loss of titre along with the precipitation of the serum is relatively small, about 10%. In general, one cc. of the serum produced 50-70 mg. of dry powder; (one mg. = 1-2 PAU). The latter is easily soluble in distilled water, in slightly acid and slightly alkaline buffers as well as in diluted acetone and alcohol. It is of importance that the precipitate results as a very fine-grained one, coarse-grained serum powder being very slowly and not completely soluble.

The antigonadotropic factor was further concentrated by salting out procedures with saturated ammonium sulphate solution (SAS). Using serum or adequate solutions of acetone dry powder in distilled water (60 mg. in one cc.) we were able to precipitate the antigonadotropic factor almost quantitatively at 48% SAS (pseudoglobulin fraction). The fractions below this concentration were found to contain only slight amounts of the antigonadotropic factor. Using a more concentrated solution of the antigonadotropic factor (60 mg. dry powder in 0.5 cc.) we were able to precipitate it already in the globulin fraction at 28.6% SAS.

In analogy to the preparation from the blood we used the following method to determine the content of the antigonadotropic factor in the tissues: The tissues were cut into very small pieces, pounded in a mortar with sea-sand, and finally extracted in the shaking-machine for 4 hours (after addition of 10 times the amount of distilled water). After being centrifuged, the dim rose-colored viscid extract was filtered and then precipitated with 4 times the volume of acetone. The precipitate is washed 6 times with acetone, while constantly being stirred on a Buchner or Seitz filter, then dried 3 times with ether, and finally air is drawn through for 5 minutes. The resulting fine white powder is placed into the exsiccator over night and is once more extracted on the following day. The extraction takes half an hour respectively in the shaking-machine; at first 4 times with distilled water, then twice with an alkaline glycocoll-buffer (pH = 9.6) and finally twice with an acid glycocoll-buffer (pH = 3.5). After having combined these extracts we have a clear brownish solution, which may be reprecipitated into a fine-grained acetone dry powder. As mentioned above in connection with the serum, this powder is easily and completely soluble in any required amount of distilled water.

Urine was assayed as well by means of this concentration method for its content of antigonadotropic factor.

We studied the following organs as to their capacity of forming and storing antihormone: ovaries, testes, liver, spleen, musculature, urine. The results obtained were as follows:

The serum of a rabbit treated daily with 250 RU prolan for 26 weeks had a maximal titre of 150 PAU per cc.

Liver, spleen and musculature of the experimental animal contained nothing of the antigonadotropic factor.

The urine of the experimental animal contained nothing of the antigonadotropic factor.

Castrated male and female rabbits formed the antigonadotropic factor in their blood in the same manner as normal animals.

9369

The Antigonadotropic Factor. Species Specificity and Organ Specificity.

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There are contradictions to be found in literature as to the specificity of the antigonadotropic factor. Bachmann, Collip and Selye,[1] Fluhmann,[2] Meyer and Gustus,[3] Brandt and Goldhammer,[4] Twombly,[5] Sulman,[6] Thompson,[7] hold that the species specificity of the antigonadotropic factor is proved. Gegerson, Clark and Kurzrok,[8] Rowlands,[9] Parkes and Rowlands,[10] however, presented evidence against the species specificity. Recently Collip[11] reported similarly. However, the latter workers had other test objects (inhibition of ovulation in the mated rabbit or of the oestral cycle in the normal rat) than the former,[1-7] and than we did. Fluhmann,[2] Brandt and Goldhammer,[4] Gegerson, Clark and Kurzrok,[8] Parkes and Rowlands[10] are opposed to the presence of an organ specificity. Selye, Collip and Thompson,[12] however, favor it. To investigate this we performed the following experiments. We used the technique described in our first report,[13] that of the exact titration of the gonadotropic hormone against adequate amounts of the antigonadotropic factor in infantile female rats and mice.

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[1] Collip and Selye, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

[2] Fluhmann, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1595; *Am. J. Obst. Gynec.*, 1935, **30**, 584.

[3] Meyer and Gustus, *Science*, 1935, **81**, 208.

[4] Brandt and Goldhammer, *Z. f. Immunitaetsforschung*, 1936, **88**, 79.

[5] Twombly, *Endocrinology*, 1936, **20**, 311.

[6] Sulman, *J. Exp. Med.*, 1937, **65**, 1.

[7] Thompson, PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 634.

[8] Gegerson, Clark and Kurzrok, PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 193.

[9] Rowlands, *Proc. Roy. Soc. London*, 1937, B, No. 824, **121**, 517.

[10] Parkes and Rowlands, *J. Physiol.*, 1936, **88**, 305; *Lancet*, 1937, p. 924.

[11] Collip, *Canad. Med. Assoc. J.*, 1937, **36**, 199.

[12] Selye, Collip and Thompson, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487, 566.

[13] Zondek and Sulman, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

While studying the species specificity and organ specificity it was essential to pay attention to the minimal and maximal values. We, therefore, thoroughly titrated maximal amounts of the antigonadotropic factor (50-100-200 PAU or PSAU*) against minimal amounts of such gonadotropic factor foreign to the species or to the organ, (1-5-10 RU). In control experiments we confirmed the titre of the gonadotropic and that of the antigonadotropic factor. The results are shown in Table I.

Table I shows (Experiments 1 and 4) that the antigonadotropic sera are active against the gonadotropic preparation used for the preliminary treatment. In contrast to this 200 PSAU (Experiment No. 6) cannot inactivate one RU of gonadotropic hormone from another species, *i. e.*, an antigonadotropic serum has less than 0.5% of its effectiveness if used against a heterologous gonadotropic factor.

In further experimental series (Table II) we studied the organ specificity of human gonadotropic hormone derived from a different starting material (urine, blood, pituitary). In Table II we find unmistakably the explanation of the extent of the organ specificity of the antigonadotropic factor. The effectiveness of the pregnancy-urine-prolan "antiserum" against the homologous "antigen" is demonstrated in Exp. 1. Exps. 2 and 4 show that the effectiveness of this "antiserum" against blood prolan or pituitary prosylan of the same species amounts to only about 7% of its titre against pregnancy-urine-prolan; *i. e.*, compared with the strict species specificity there is only a relatively high organ specificity. It was only by using highly concentrated "antisera" and by titrating exactly ascertained large doses of the antigonadotropic factor against such doses of gonadotropic substance which were certainly effective and exactly titrated that we arrived at these results.

The following experiment represents a further indication as to the species specificity of the antigonadotropic factor. Four female rats got 100 RU of prolan twice weekly subcutaneously during a period of over a year. In 2 of the animals the antigonadotropic titre of the blood was examined. Because of the non-intense preliminary treatment (only twice a week) it was low (20 PAU per cc.). In the

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* 1PAU = prolan anti-unit is the minutest amount of the antigonadotropic factor required to annihilate the gon. effect of 1 RU prolan in the immature female rat. 1PSAU = 1 prosylan anti-unit is similarly defined. N.B. At least 10 units should be assayed in a test rat. We call prolan the gonadotropic hormone derived from pregnancy urine, prosylan that containing, in addition to prolan, synprolan (synergetic factor), *cf.* Zondek, B., *Act. Obst. et Gyn. Scandin.*, 1935, **15**, 1.

TABLE I.
Species Specificity of the Antigonadotropic Factor.

Exp. No.	Antigonadotropic factor against	Anti-units PAU-PSAU	Gonadotropic factor from	Rat Units RU	HVR I-III (gonadotropic reaction)	Result: antigonadotropic effect
1.	human pregnancy urine prolan†	100 PAU	human pregnancy urine prolan†	100 RU	neg.	% 100
2.	,, ,,	100 PAU	pregnant mare's serum prosylan†	5 RU	pos.	< 5
3.	,, ,,	100 PAU	bovine hypophyseal prosylan†	5 RU	,,	< 5
4.	pregnant mare's serum prosylan†	200 PSAU	pregnant mare's serum prosylan†	200 RU	neg.	100
5.	,, ,,	200 PSAU	human pregnancy urine prolan†	5 RU	pos.	< 2.5
6.	,, ,,	200 PSAU	bovine hypophyseal prosylan†	1 RU	,,	< 0.5

TABLE II.
Organ Specificity of the Antigonadotropic Factor.

Exp. No.	Antigonadotropic factor against	Anti-units PAU	Gonadotropic factor from	Rat Units RU	HVR I-III (gonadotropic reaction)	Result: antigonadotropic effect
1.	human pregnancy urine prolan†	100	human pregnancy urine prolan†	100 RU	neg.	% 100
2.	same	100	human pregnancy blood prolan‡	7 RU	,,	> 7
3.	,,	100	same	15 RU	pos.	< 15
4.	,,	100	human antepituitary prosylan‡	7 RU	neg.	> 7
5.	,,	100	same	15 RU	pos.	< 15

‡ The pregnancy-blood-prolan was prepared as an acetone dry powder, the hypophysis prosylan according to the method of Van **Dyke and Wallen-Lawrence.**

other 2 animals one ovary respectively was extirpated. Each of them averaged 14 mg. in weight and in the histological examination they showed a few moderately enlarged follicles as well as some old vascularized corpora lutea. The ovaries, therefore, revealed only a weak reaction upon prolonged prolan treatment. But when we injected 100 MU of antex† (pregnant mare's serum prosylan) the remaining ovary reacted with an increase in weight up to 7 times the amount (100 mg.) and with enormous swelling of the follicles, blood spot formation and luteinization. (HVR I, II and III.) This experiment also suggests the species specific behavior of the antigonadotropic factor: the ovaries did not react further with gonadotropic hormone of human origin, they did, however, react strongly with hormone of animal origin.

No doubt in every organism not preliminarily treated there are in addition to these specific antigonadotropic factors, non-specific gonadotropic antagonists which may be looked upon as antihormones in the strictest sense of the word. According to Evans, *et al.*, such an antagonist is to be found in the pituitary,[14] according to Engelin in the epiphysis,[15] and according to Hoffmann, *et al.*, in the adrenals.[16] Prompted by the results of these workers we performed the following experiments:

We injected into an infantile female rat an aqueous extract of 200 mg. acetone dry powder from bovine epiphyses. The following day the injection was repeated and 10 RU of prolan added. The gonadotropic reaction (HVR) as usual was read 120 hours later. No inhibition of the gonadotropic reaction could be found. We repeated the experiments with 2 ampules of Epiphysan§ which had the same negative result. There are, consequently, no observable amounts of gonadotropic antihormone in the epiphysis.

We injected into an infantile rat 1.0 Eschatin|| (adrenal cortex extract) and on the following day 0.5 Eschatin + 10 RU of prolan. A similar experiment was started with half the dose of Eschatin. The result after 120 hours was in 2 of the experiments complete

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† We used prolan from pregnancy urine of the I. G Farben-Ind.; antex, from pregnant mare's serum of the Løvens Kemiske, Kopenhagen; preglandol, from bovine pituitaries of Hoffmann-La Roche, Basel. We are indebted to the above firms for kindly supplying the preparations.

[14] Evans, Korpi, Pencharz and Simpson, *Univ. Calif. Publ. Anat.*, 1936, **1**, 237.

[15] Engelin, *Klin. Wschr.*, 1935, **14**, 970; *Wien. Klin. Wschr.*, 1935, **48**, 1160.

[16] Hoffmann *et al.*, *Klin. Wschr.*, 1937, **16**, 79.

§ We are indebted to the firm Gideon Richter, Budapest, for kindly supplying the preparations.

|| We are indebted to the firm Parke, Davis & Co. for kindly supplying the preparations.

inhibition of the gonadotropic reaction; in 4 further experiments, however, no such inhibition was found. We repeated the experiment with the same doses of Cortigen§ and did not find inhibition of the gonadotropic reaction. The adrenals, therefore, do not contain noticeable amounts of antigonadotropic hormone.

We hold that the above experiments explain the contradiction in literature as to the species specificity and organ specificity of the antigonadotropic factor. These contradictions are based upon the fact that the majority of the workers approached the problem from the qualitative side, not from the quantitative one. Those workers[9-11] who are opposed to the species specificity inhibited the normal oestral cycle in the rat or the ovulation in the mated rabbit, etc., by applying comparatively enormous doses of the antigonadotropic factor. The rat, to bring about its oestrous phase, produces only about one RU of gonadotropic factor, consequently the experiments of the workers[9-11] only prove that large amounts of antigonadotropic factor (especially if applied daily) are able to annihilate the effect of one RU of heterologous (foreign to the species) prosylan. Without doubting the correctness of these investigations we approached the question quantitatively, and found that antigonadotropic sera show less than 0.5% of their effectiveness against a heterologous gonadotropic factor, involving a loss of titre by at least 99.5%. Even if the conclusions drawn by the workers[9-11] from their experiments are fully correct as to their theoretical meaning (that there is no absolute species specificity) our quantitative experiments reveal practically that the species specificity is enormous (more than 99.5%). On these grounds we think we may be permitted to state: The antigonadotropic factor works specifically to the species.

Regarding the organ specificity we draw the conclusion: The antigonadotropic factor against human pregnancy-urine-prolan reveals only 7% of its titre if used against human pregnancy-blood-prolan or against prolan of human pituitary origin, *i. e.*, a loss of 93% of its effectiveness.

So in a serological sense every prolan preparation has a different antigenic structure. Another difference between the gonadotropic preparations derived from different sources is variation in time necessary for the formation of the antigonadotropic factor. Thus prolan derived from human pregnancy urine induces the formation of antiprolan in the rabbit after one month's treatment; gonadotropic hormone derived, however, from human or mare's pregnancy blood requires about 3 times as long. We believe the quantitative method of investigation proposed by us to be suitable for giving a closer insight into the difference in structure of the gonadotropic factors derived from different sources.

The property of a strict species specificity and of a relatively high organ specificity of the antigonadotropic factor gives us a clue as to the mechanism of the antigonadotropic effect. It is certainly not a humoral antagonistic effect (as *e. g.*, to be found in the insulin-adrenalin-contra-mechanism) but a process having its effective point directly and specifically in the gonadotropic preparation and rendering the preparation itself ineffective biochemically. We shall furthermore report experiments leading to the supposition that the gonadotropic hormone irreversibly breaks down and is destroyed by the homologous antigonadotropic factor.

Basing our deductions upon the above investigations we have to differentiate between 2 groups of gonadotropic antihormones:

First Group. Antigonadotropic factors formed in the blood following protracted treatment with a heterologous gonadotropic hormone (preferably daily subcutaneous or intravenous application). The formation of these antigonadotropic factors is exclusively connected with the blood, independent of the species of animal, sex and the presence of sexual functions (castration). These antigonadotropic factors are likely to take their effect by splitting up the gonadotropic hormone. They reveal less than 0.5% of their effectiveness if used against a heterologous gonadotropic factor, 7% of their effectiveness if used against a homologous gonadotropic factor, which is, however, foreign to the organ.

Second Group. Gonadotropic antagonists of the normal non-treated organism. Such antagonists are said to occur in the pituitary and in the adrenals of normal animals not having been preliminarily treated. The mechanism of their effectiveness is hormonal, unspecific to species and organ. Their recognition in the pituitary gland and in the adrenals is rendered difficult because these organs produce simultaneously several hormones. Thus does the pituitary liberate hormones inducing the gonadotropic reaction and the adrenals such hormones advancing it. In the epiphysis we could not find any noticeable amounts of the gonadotropic antagonistic factor.

9370

Use of Sulphanilamide (Para Amino Benzene Sulphonamide) in Experimental Poliomyelitis.*

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The effectiveness of sulphanilamide (para amino benzene sulphonamide) in the treatment of certain bacterial infections[1] suggested its trial in the treatment of a virus infection, experimental poliomyelitis. Though no reports of the use of sulphanilamide in any virus diseases have appeared, Long[2] has observed no improvement of concurrent common colds in patients receiving the drug for various streptococcal infections. Marshall and his associates[3] have shown that sulphanilamide diffuses readily through the tissues of the body, and into the cerebrospinal fluid, where it reaches a level slightly lower than that of the blood stream. Two bacterial infections of the meninges, Beta hemolytic streptococcal meningitis[4] and meningococcal meningitis[5] have responded well to sulphanilamide therapy.

Two series of *Macacus rhesus* monkeys were used in these experiments. The first 4 monkeys were inoculated by the intranasal route with 0.5 cc. of 20% fresh virus cord suspension in each nostril on 2 successive days; two of these monkeys received subcutaneous injections of 50 cc. of a 1% saline solution of sulphanilamide‡ (an amount equal proportionately to twice the maximal therapeutic human dose) twice daily, begun 3-4 hours prior to the first inoculation of the virus suspension; two were used as controls. In the second group, 6 monkeys received one cc. each of 15% virus cord suspen-

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* Work supported by the President's Birthday Ball Commission for Infantile Paralysis Research.

† Fellow in Medicine, The Johns Hopkins University.

[1] Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32. A full bibliography is given here.

[2] Long, P. H. Personal communication.

[3] Marshall, E. K., Jr., Emerson, Kendall, Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 12.

[4] Arnold, J. G., Jr., *Ann. Int. Med.*, 1937, **10**, 8; Schwentker, F. F., Clason, F. P., Morgan, W. A., Lindsay, J. W., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 4.

[5] Schwentker, F. F., Gelman, S., and Long, P. H., *J. A. M. A.*, 1937, **108**, 17.

‡ Marshall (in work to be published) has shown that the blood sulphanilamide level 4 hours after one such injection is 12.7 mg.%, and after 8 hours, 11.1 mg.%. Practically all this is in the active uncombined form (though the urine contains 30-70% of the inactive acetylated compound). Such a concentration lies within the supposedly effective range of the drug in human blood.

sion in each nostril on 2 successive days; 4 were given sulphanilamide as above, while 2 control animals received 50 cc. of normal saline subcutaneously twice daily. Tables I and II show the course of these monkeys.

TABLE I.
First Series.

No. of Monkey	Day of Onset of Paralysis	Complete Paralysis	Day of Death	Remarks
				Treated with 50 cc. 1% sulphanilamide in saline s.c. b.i.d.
61	11	15	17	
62	8	—	—	Moderate flaccid paralysis of right arm disappeared completely by 12th day. Animal sacrificed on 17th day.
				Untreated Controls
63	12	14	22	Received 50 cc. normal saline s.c. b.i.d. 17th to 20th day.
64	8	9	9	Animal sacrificed when completely paralyzed and moribund.

s.c. = subcutaneously.

TABLE II.
Second Series.

No. of Monkey	Day of Onset of Paralysis	Complete Paralysis	Day of Death	Remarks
				Controls—Given 50 cc. normal saline s.c. b.i.d.
70	12	—	—	Animal showed severe paralysis of both legs and moderate paralysis of the arms. From the 27th day on he gradually gained in vigor, with slight decrease of paralysis.
71	9	10	17	
				Treated with 50 cc. 1% sulphanilamide in saline s.c. b.i.d.
72	10	14	24	Animal sacrificed when completely paralyzed and moribund.
73	9	12	19	
74	10	11	18	
75	10	18	24	When unable to feed themselves, this monkey and the others of this series were tube-fed twice daily with 200 cc. of milk containing egg and sugar.

s.c. = subcutaneously.

It is seen that in the 2 series of experiments 2 monkeys survived, one treated animal, after transient paralysis, and a control animal showing marked, but very gradually diminishing, paralysis. Such survivals are not uncommon in untreated animals following inoculation by the intranasal route. In the other monkeys, as tabulated, there is no significant difference in the time of onset of paralysis, in the time of occurrence of complete paralysis of the extremities, or in the day of death, between the 2 groups. The results of these experiments fail to indicate any therapeutic value of sulphanilamide in experimental poliomyelitis.

9371 P

Beneficial Effect of Non-Saponifiable Fraction of Soy Bean Oil on Chicks Fed a Simplified Diet.

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Pappenheimer and Goettsch[1] have described a syndrome in chicks fed certain simplified diets. The syndrome was characterized by paralytic symptoms, and post mortem examination revealed definite lesions in the cerebellum. Recently Goettsch and Pappenheimer[2] stated that protection against the syndrome ("nutritional encephalomalacia") was afforded by the non-saponifiable fraction of certain vegetable oils, including soy bean oil.

It has been found possible to obtain confirmatory evidence of a beneficial effect of the non-saponifiable matter of soy bean oil when fed to chicks receiving a simplified diet. Crude soy bean oil* was treated as follows: To 500 cc. of boiling methyl alcohol (freshly distilled from potassium hydroxide) were added in order 1250 gm. of potassium hydroxide and 1000 gm. of crude soy bean oil. Heating was continued under reflux for one hour, whereupon 2500 cc. of boiling water was added. When cool, the resulting solution was first saturated with 3500 cc. of peroxide-free ether (prepared by shaking with 5% aqueous stannous chloride, until a sample gave no color with a colorless ferrous sulfate + potassium thiocyanate solution, and distilling), then extracted with 5 2-liter portions of the same solvent. Throughout the entire process, all apparatus was flushed with natural gas. The extracts were combined, washed first

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[1] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.

[2] Goettsch, M., and Pappenheimer, A. M., *J. Biol. Chem.*, 1936, **114**, 673.

* Kindly supplied by Allied Mills, Peoria, Ill., through the cooperation of Dr. E. M. Hasbrouck.

TABLE I.
Protective Effect on Chicks Afforded by Soy Bean Oil and Non-saponifiable Matter of Soy Bean Oil. Results Taken at 28 Days.

Supplement to basal diet	No. of birds	No. developing definite paralysis	No. dying without paralysis being observed
None	30	16	6
Ether solution of non-saponifiable matter of soy bean oil corresponding to 15% of original oil	28	0	1
10% of soy bean oil	25	0	1

with 5% sulfuric acid, then with water until neutral, concentrated and used to supplement the diet described below.

The basal diet consisted of corn starch, 65%; fat-extracted whole sardine meal,† 25; rice bran extract,[3] 7; whey adsorbate,[4] 2; alfalfa hexane extract,[5] equivalent to 0.5% of alfalfa meal; cod liver oil, 1. In some experiments a purified antihemorrhagic concentrate, kindly supplied by Dr. H. J. Almquist, replaced the alfalfa hexane extract without perceptible alteration of the results. Single-comb White Leghorn chicks were placed on this diet at hatching. On the basal diet, paralytic symptoms, accompanied by loss of weight and shortly followed in most cases by death, appeared at 20 days of age. By the 28th day, the results summarized in Table I were obtained.

The chicks on the supplemented diets appeared normal, but were definitely below normal weight. This observation of the protective effect of the non-saponifiable fraction of soy bean oil is in accord with the results of Goettsch and Pappenheimer.[2]

9372 P

Non-Transmissibility *in utero* of Trichinosis in the Rat.

Lloyd Catron. (Introduced by Carl V. Weller.)

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For several weeks after the maturation of ingested larvae of *Trichinella spiralis* in the intestine, those of the next generation enter the circulating blood of the host. Although the presence of migrating larvae in various organs, tissues and body fluids has been observed repeatedly, studies of pregnant hosts, such as the rat, guinea pig, rabbit, hog and man, have indicated that intrauterine transmission of trichinosis from mother to fetus does not occur. Roth,[1] however, recently reported the recovery by digestion of a few larvae in 2 out of 5 litters of guinea pigs born of mothers infected during pregnancy. Previous investigations have dealt with comparatively small groups of animals, and little importance has been attached to the stage of gestation at which mother animals have been infected.

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† Furnished by the F. E. Booth Co., San Francisco, by the courtesy of Mr. T. D. Sanford.

[3] Lepkovsky, S., and Jukes, T. H., *J. Biol. Chem.*, 1935, **111**, 119.

[4] Lepkovsky, S., and Jukes, T. H., *J. Biol. Chem.*, 1936, **114**, 109; Jukes, T. H., *J. Biol. Chem.*, 1937, **117**, 11.

[5] Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

Young adult white rats weighing from 180 to 280 gm. were fed by stomach tube the larvae of *Trichinella spiralis* obtained by digestion of infected rats in artificial gastric juice.[2] The time of inoculation varied from one day before, to 15 days after, copulation. The presence of infection in all mother rats was confirmed by subsequent microscopic examinations of their diaphragms and other skeletal muscles. Since migration of young larvae begins about one week after infection and usually continues for 2 weeks, larvae were migrating in various rats during all except the earliest stages in the maturation of the placenta and of fetal development of muscle. Attempts to obtain copulation of female rats 2 to 7 days after infection were unsuccessful. Twenty-one females were fed 15 larvae per gm. of body weight, an amount sufficient to cause an infection of moderate severity. To 2 groups of 3 rats each were fed 35 and 40 larvae respectively per gm. of body weight. Most of the offspring of rats fed 15 larvae per gm. were of usual size and weight, and developed normally. The few which did not appear perfectly healthy were included among those examined for trichinosis. Most of the young of heavily infected rats died. Those living several days soon became underweight, scrawny and feeble as compared to normal rats of similar age. None was observed to have diarrhea.

To determine whether migrating larvae entered fetal tissues, microscopic examinations were made of fresh teased and pressed preparations and of sediments from centrifuged washings of serous cavities. Also several sections from each of 6 to 10 serial blocks were prepared from each young rat examined; in this way practically all organs and tissues were searched. Sections were made of the 9 fetuses and placentas from one moderately infected mother killed on the 21st day of gestation. Similar material was examined from the 8 fetuses and placentas of each of 2 heavily infected mothers dying on the 17th and 18th day of gestation. In the 25 placentas examined, no larvae were found in either fetal or maternal portions. Of animals born, microscopic examinations were made on 26 from 5 litters which were stillborn or dying at ages up to 20 days, and on 8 from 6 litters killed at from one to 20 days of age. Material was taken from 7 to 38 days after infection of the mothers. In no instance were trichina larvae found in fetal tissues or fluids.

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[1] Roth, H., *Acta Path. et Microbiol. Scand.*, 1935, **12**, 203.

[2] McCoy, O. R., *Am. J. Hyg.*, 1931, **14**, 484.

Digestion of the skinned and eviscerated carcass in a solution of hydrochloric acid and pepsin is useful only for the recovery of larvae which have become encysted in skeletal muscle. At 60 to 70 days of age, one male and one female from each of 17 litters of mothers fed 15 larvae per gram were digested. The mothers had been infected at the following times: 15, 14 (2), 12, 11 (2), 10 (2), 8, 7, 5 (2), 4, 3 (2), and one days after, and in one instance one day before, copulation. No larvae were found.

These results indicate that in the white rat trichina larvae circulating in the maternal blood are not capable of crossing the placental barrier into the fetal circulation and tissues. We have found no larvae in the sections of placentas examined. Larvae also have not been found to migrate from the peritoneal cavity of the mother rat into the amniotic sac or embryo proper. Finally, our results support the view that trichinosis is not transmitted by way of the milk or feces, as the young rats were suckled by their own mothers and were caged with them.

9373

Effect of Acute Infection on Iodine Number of the Phospholipid Fatty Acids.*

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There is a definite lowering of the values for the plasma lipids at the height of an acute infection as compared with the levels obtained during convalescence.[1] Fever *per se* is not responsible for the fall of the various lipid constituents of the blood plasma. Diet does not have any demonstrable influence on the results. The drop in the total cholesterol values is due almost entirely to a marked decrease in the ester cholesterol[2] and this condition holds true for infants as well as children.[3] The fall in the total fatty acid values is accompanied by low iodine absorption values yielding iodine numbers of the serum fatty acids which are significantly lower during the febrile period of the disease than during the afebrile period of convalescence.[4, 5] The plasma phospholipids do not in all cases drop immediately to low levels after the onset of the acute illness. In some instances, values above the normal range have been obtained early in the disease. The behavior of the phospholipid fatty acids in relationship to the variations in the phospholipids in acute infections of children has not been recorded.

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* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

[1] Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Child.*, 1935, **49**, 658.

Eighteen children ranging in age from 13 months to 15 years were chosen for this study. Nine of the subjects had upper respiratory infections with acute otitis media, acute bronchitis, or cervical adenitis, while the remaining 9 had pneumonia with no complications. All the blood samples obtained during and after the height of each infection were drawn between 12 and 16 hours after a meal. The first blood sample was collected in 8 of the cases before the end of the second day of the febrile period of the disease and in the remaining 10 patients between the sixth and seventh day of the illness. The second blood sample was collected in all children between the seventh and ninth days of convalescence. Bloor's method[6] as modified by Boyd[7] was employed to obtain the total phospholipid content of the blood plasma and the Rosenmund-Kuhnhenn method[8] as modified by Page, Pasternak and Burt[9] was utilized to determine the iodine absorption values of the phospholipid fatty acids.

The results are summarized in Table I.

There was with only 2 exceptions a definite fall in the plasma phospholipid values in the group of children in whom the first blood sample was obtained early in the disease. This may indicate that the rise in total phospholipids observed by Boyd[10] in acute infections of the adult must occur in the child very soon after the onset of the

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[2] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1324.

[3] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 10.

[4] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1326.

[5] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 12.

[6] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[7] Boyd, E. M., *J. Biol. Chem.*, 1933, **101**, 323.

[8] Rosenmund, K. W., and Kuhnhenn, W., *Z. f. unter. d. Nahr. d. Nahr. u. Genuss.*, 1923, **46**, 154.

[9] Page, H. H., Pasternak, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

[10] Boyd, E. M., *Canadian Med. Assn. J.*, 1935, **32**, 500.

TABLE I.

Iodine Numbers of the Phospholipid Fatty Acids in Infections of Upper Respiratory Tract and in Pneumonia.

Case No.		Plasma Phospholipids Mg. per 100 cc. serum		Iodine No.	
A	B	A	B	A	B
		Febrile period of disease.			
1	9	95	125	146	57
2	10	85	98	146	70
3	11	159	124	88	67
4	12	181	60	105	76
5	13	124	64	131	52
6	14	111	93	153	84
7	15	76	121	161	51
8	16	72	122	147	87
	17		106		67
	18		101		62
Aver.		112	101	134	67
		Afebrile period or convalescence			
1	9	175	170	120	84
2	10	180	174	115	83
3	11	116	179	51	110
4	12	136	154	77	149
5	13	182	103	116	87
6	14	184	177	120	113
7	15	162	164	109	110
8	16	121	139	102	98
	17		221		85
	18		167		86
Aver.		157	165	101	100

A—First blood sample collected second day of febrile period.
B—First blood sample collected sixth or seventh day of febrile period.

infection. The decrease in the serum phospholipids was not accompanied with a fall in the iodine absorption values. On the contrary, the iodine numbers were higher than normal at this stage of the disease. The ratio of saturated to unsaturated phospholipid fatty acids referred to by Sinclair[11] was certainly greatly disturbed by the acute infection, there being a relative increase in the unsaturated fatty acids probably in response to an increase in the intensity of the fat metabolism during the febrile period.

The plasma phospholipid values were consistently low in the other group of children in whom the first blood sample was obtained toward the end of the height of the disease. This condition was accompanied by a marked fall in the iodine absorption of the phospholipid fatty acids yielding low iodine numbers. The desaturation may be entirely due to the rapid utilization of the unsaturated fatty acids by the altered metabolic processes present during an infection. There is, however, an additional factor which could play a rather significant rôle. The observation has been made that certain toxic substances such as cobra venom when mixed with lecithin cause it to lose its unsaturated fatty acid radicle.[12] The resulting lecithin compound contains a relatively high proportion of saturated fatty acids. The question thus arises as to whether the bacterial toxins of an acute infection could have the same influence on the plasma phospholipids. Most logical at the present time is the assumption that the intensity of the fat metabolism during an acute infection and the presence of the bacterial toxins in the blood may both be responsible for the ultimate fall in the plasma phospholipids to abnormal levels with an associated extensive loss of their unsaturated fatty acids. Should this actually be found to occur, it would give the phospholipids an important part in the phenomena of immune reactions.

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[11] Sinclair, R. G., *Physiological Rev.*, 1934, **14**, 351.

9374 P

Photoelectric Spectrophotometry Applied to Studies in Fat Metabolism.*

E. S. MILLER AND G. O. BURR.

From the Department of Botany, University of Minnesota.

Recently new methods have been applied for following the rate of movement and metabolism of fats. Schoenheimer and Rittenberg[1] have published a series of papers on the use of deuterium as an indicator in fatty acid metabolism. Sinclair and coworkers[2] have made use of elaidin as an indicator of rate of movement and metabolism of fats in various rat tissues.

The common fats and fatty acids show very low absorption of light from 2000 to 8000 A.U., except for a weak band at 2300 A.U.[4] However, there are 2 exceptional cases. α-Eleostearic acid of tung oil has a very strong maximum at 2700 A.U.[3] Moore[4] has shown that linseed oil fatty acids become strongly "absorptive" on

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[12] Peters, John P., and Van Slyke, Donald, *Quantitative Clinical Chemistry*, Williams and Wilkins Company, Baltimore, 1931, Vol. 1.

* Aided by grants from the Rockefeller Foundation, the Medical Research Fund of the Graduate School of the University, and the National Live Stock and Meat Board.

[1] Rittenberg, D., and Schoenheimer, R., *J. Biol. Chem.*, 1937, **117**, 485.

[2] McConnell, K. P., and Sinclair, R. G., *J. Biol. Chem.*, 1937, **118**, 123.

[3] Dingwall, A., and Thomson, J. C., *J. Am. Chem. Soc.*, 1934, **56**, 899.

[4] Moore, T., *Biochem. J.*, 1937, **31**, 138.

prolonged saponification. These acids are digestible and cure fatty acid deficiency symptoms. It has also been shown that highly unsaturated fatty acids are changed to an absorptive form by the cow and secreted in milk.[5]

Using apparatus similar to that described by Hogness, Zscheile and Sidwell,[6] small amounts of a strongly absorbing lipid can be determined in the presence of the usual body lipids. This method offers the advantage of speed and accuracy of determination on very small samples. Less than 10 mg. of a lipid are required.

Tung oil was chosen for the first work because of its ready digestibility and very high absorption. At 2700 A.U. $E^{1\%}_{1\,\text{cm.}} = 1400$ in the specimen of oil used. At the same wave length adipose tissue of a rat has an $E^{1\%}_{1\,\text{cm.}}$ of less than one. Therefore, a very small amount of the tung oil acids deposited in a rat tissue is readily detected.

Three mature rats on stock diet were starved over night (18 hours) and fed at 9 a. m. 10 gm. of the same stock diet mixed with 1 gm. of tung oil. Uneaten food was removed from the cage in the afternoon and total oil consumption calculated. Regular stock diet was put in the cage so that there was no starvation following oil intake. One rat was killed at 9 a. m. each succeeding morning, that is, at 24, 48 and 72 hours after the first intake of tung oil. Table I summarizes the data.

TABLE I.

Case	Rat No.	Body wt. g.	Tung oil consumed g.	Time killed after first oil intake hr.
1	36194	180	0.9	24
2	36210	222	0.9	48
3	36212	189	0.64	70

At autopsy the blood, liver, kidneys, brain, skin and adipose tissue were collected. Solid tissues were ground with glass and the fat removed by prolonged alcohol-ether extractions. Solvents were removed *in vacuo*, true lipids taken up in petroleum ether, which was then removed *in vacuo* in a tared flask. The samples were made to volume in purified ether and aliquots used for the spectroscopic studies. Some results are summarized in Figs. 1 to 6.

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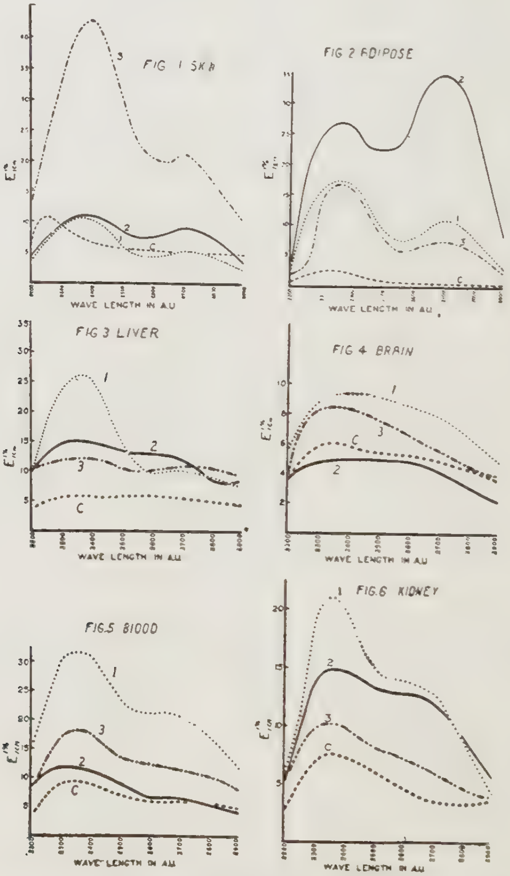
[5] Dann, W. J., Moore, T., Booth, R. L., Golding, J., and Kon, S. K., *Biochem. J.*, 1935, **29**, 138.

[6] Hogness, T. R., Zscheile, F. P., Jr., and Sidwell, A. E., Jr., *J. Phys. Chem.*, 1937, **41**, 379.

Full interpretation of the curves is not possible at this time. First, it should be pointed out that the animal very quickly changes eleostearic acid, causing a loss of the band at 2700 A.U. and a rise in absorption at 2350 A.U. This fact must be considered in evaluating the data.



FIGS. 1-6.

Absorption curves of lipids extracted from six tissues of three experimental rats and a control. Curves numbered 1, 2 and 3 represent rats killed 24, 48, and 70 hours respectively after consumption of the first tung oil. C is the control on stock diet, but with no tung oil.

However, certain very interesting differences in tissues are at once apparent. According to the rapidity with which the 2700 A.U. band disappears during the first day, the tissues fall in the following order: liver, kidney, blood, adipose and skin. Adipose tissue received a large amount of unchanged acid (Fig. 2, curve 2) during the second day, which was largely gone by the third day. The new acid with high absorption at 2350 A.U. persisted through the third day. The skin received almost none of this material for 2 days, but on the third day large amounts of the changed acid were deposited (Fig. 1, curve 3). The brain showed a small but appreciable response to tung oil feeding. The order of the curves is the same as for blood, which suggest that the immediate blood supply may control the results; but the amount of blood present can not account for all of the absorptive acid, as the brain lipids were several times the total blood lipids.

Conclusions. Eleostearic acid is quickly changed to a new acid *in vivo.* Distribution and metabolism of this acid has been followed by spectroscopic analysis and large differences found among several tissues.

9375 P

Reaction of the Supraoptic Nucleus to Hypophysectomy.*

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The origin and significance of the hypothalamico-hypophyseal neural system are still controversial. Keller, Noble and Hamilton[1] and Mahoney and Sheehan,[2] who interrupted these fibers in the infundibulum, rather minimize their rôle. On the other hand, Ranson and co-workers seem to have established a very close connection between lesions in this system and at least diabetes insipidus.

The origin is generally regarded as being the paired supraoptic nuclei, 2 more dorsally situated paraventricular (filiform) nuclei and some less definitely localized cells in the tuber cinereum. In the dog retrograde degeneration of the supraoptic nuclei 10-16 days after destructive lesions in the processus infundibuli and infundibulum was noted by Kary,[3] Lewy,[3] Maiman[3] and Broers.[4] Hare[5] reports a loss of more than half of these cells in hypophysectomized dogs. In the cat, Fisher, Ingram, Hare and Ranson[6] further showed that unilateral destruction of the neural lobe of the hypophysis or electrolitic interruption of the nerve fibers before entering the infundibulum causes atrophy of the homolateral supraoptic nucleus. They produced retrograde degeneration also in monkeys.[7] Changes in other nuclei apparently have not been clearly demonstrated. An increase in neuroglia cells has been mentioned.

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* Aided by a grant from the Research Fund, Graduate School, University of Minnesota.

[1] Keller, A. D., Noble, W., and Hamilton, J. W., Jr., *Am. J. Physiol.*, 1936, **117**, 467; PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 794; Keller and Noble, *Proc. Am. Physiol. Soc.*, 1936, **48**, 90.

[2] Mahoney, W., and Sheehan, D., *Brain*, 1936, **59**, 61.

[3] Kary, K., *Virchows Arch. f. path. Anat. u. Physiol.*, 1924, **252**, 734; Lewy, F. H., *Zentralb. f. d. ges. Neurol. u. Psych.*, 1934, **37**, 398; Maiman, R. M., *Z. f. d. ges. Neur. u. Psych.*, 1930, **129**, 666.

[4] Broers, H., *Diss. Inaug. Kemink en Zoon*, Utrecht, 1932.

[5] Hare, H., *Proc. Am. Physiol. Soc.*, 1937, **49**, 70.

Mahoney and Sheehan,[2] as a result of placing silver clips on the infundibulum of dogs and monkeys, could see no definite evidence of retrograde degeneration. In the chimpanzee, Mahoney[8] found no hypothalamic change of note 17 days after hypophysectomy. Mahoney and Sheehan found cells with pale centers and peripheral clumping of Nissl granules (features repeatedly taken as evidence of degeneration) in normal supraoptic and paraventricular nuclei. Malone[9] showed this many years ago in colored plates of these nuclei in man, monkey, lemur and cat. Greving[10] presents the same features by means of photomicrographs. We can confirm this as being normal histology in man, monkey, dog, cat, and rat. The nucleus is frequently very eccentric in the larger cells.

In the light of the above we have studied the hypothalamic nuclei following hypophysectomy in 29 albino rats. Twenty-one of these were operated on when 21 days of age (40-56 gm.) and 8 when mature (180-260 gm.). Suction through the pharyngeal route, without damage to the brain, was the method used. The animals were sacrificed from one week to 6 months later. Various amounts of anterior lobe tissue happened to be retained in some cases so that various degrees of stunting occurred.

By 10 days a very definite loss of cells in the supraoptic nucleus is apparent. By 6 months only 1/5 of the cells may be left in rats operated upon at weaning age. Many of the cells remaining are normal in appearance. Occasionally an enlarged cell is encountered and others are hyperchromatic and atrophic with pycnotic nuclei. No reaction is apparent on the part of the neuroglia. In the older group the cell loss is even greater. About 90% may disappear in

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[6] Fisher, C., Ingram, W. R., and Ranson, S. W., *Arch. Neur. and Psych.*, 1935, **34**, 124; Fisher, Ingram, Hare and Ranson, *Anat. Rec.*, 1935, **63**, 29; Ingram and Fisher, *Anat. Rec.*, 1936, **66**, 271.

[7] Ingram, W. R., Fisher, C., and Ranson, S. W., *Arch. Int. Med.*, 1936, **57**, 1067.

[8] Mahoney, W., *Proc. Am. Physiol. Soc.*, 1936, **48**, 105.

[9] Malone, F. F., *Johns Hopkins Hosp. Reports*, 1914, Monographs, N.S. No. 4.

[10] Greving, R., *Handbuch der Mikroskopischen Anatomie* (v. Möllendorff), 1928, **4**, 917.

149 days. The cells remaining are either associated with the infundibular stump still attached to the brain and the median eminence of the tuber cinereum or are part of non-hypophyseal circuits. Retrograde degeneration in the supraoptic nucleus of the rat is sufficiently extensive to leave no doubt about this nucleus being primarily concerned with the neural lobe of the hypophysis. There are indications that the ventrolateral or magnocellular portion of the paraventricular nucleus also reacts to hypophysectomy by a loss of cells, but to a lesser extent. More extensive studies along this line are being carried on by Dr. L. S. Meriwether. The technical skill of Mr. Dwight Ingle, who performed the operations is gratefully acknowledged.

9376

Augmentation of Gonad Stimulating Hormone of the Hypophysis by Copper

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In a recent report[1] it was shown that zinc sulphate in combination with antuitrin S augmented the weights of the ovaries of immature rats above that obtained with antuitrin S alone. In contrast to this, zinc sulphate had no augmentative effect when given in combination with pituitary implants. Copper, on the other hand, seems to differ from zinc in certain of these ovarian augmentative reactions. Fevold, *et al.*,[2] obtained ovulation in rabbits by intravenous injections of copper salts but failed to do so with zinc. In these rabbits the copper may have altered the response of the gonad stimulating hormones present in the blood. It, therefore, seems likely that a similar mechanism might be induced in immature rats by combinations of copper salts and pituitary implants. The data on this point are described in the present study.

The recipients were albino rats of approximately 38 gm. body weight. All donors were normal adult male rats. The pituitary implants were made in the abdominal cavity under ether anesthesia. Copper sulphate was given intraperitoneally once or twice daily in doses up to one-third of a milligram at a time in one cubic centi-

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[1] Emery, F. E., *Am. J. Physiol.*, 1937, **118**, 316.

[2] Fevold *et al.*, *ibid.*, 1936, **117**, 68.

TABLE I.
Ovarian Weights in Milligrams of Rats at 30 Days of Age.
The $CuSO_4$ represents the total amount given over the 5-day period.

Groups	No. of Rats	Pituitary Implants	$CuSO_4$	Median	Mean	Range	% Increase	Groups Compared	Mean Difference / S.E. of Difference
A	15	None	0	13.0	13.0	9 to 18	—	—	—
B	30	1 ♂	0	15.0	17.0	9 to 52	30.7	A, B	1.4
C	26	1 ♂	0.5 to 2 mg.	17.5	22.3	10 to 64	71.5	B, C	1.7
D	16	2 ♂	0	50.0	45.4	12 to 73	249.0	B, D	4.4
E	12	2 ♂	0.5 to 2 mg.	50.0	47.7	14 to 93	267.0	D, E	0.2

meter of saline solution. The ovaries were removed 5 days after the implants were made.

One normal male pituitary increased the average weight of both ovaries from 13.0 mg. (controls) to 17.0 mg. (groups A and B, Table I). Under the same treatment but with copper sulphate the ovaries weighed 22.3 mg. (group C). In this group the distribution of the ovarian weights was more even throughout the range while in those without copper (group B) in only 2 cases were the weights above 24 mg. The median like the mean was higher in the copper treated group but the differences are small and not statistically significant. The mean difference divided by the standard error of the difference is only 1.7. One male rat pituitary gland is quite a minimal stimulus and positive vaginal smears are produced without much change in ovarian weight. This we have previously noted[1, 3] and it is evident here in groups A and B.

When the stimulus was doubled and 2 rat pituitary glands implanted in the body cavity, the ovaries were significantly larger than when only one pituitary was given (groups B and D). Here again copper was without effect, the medians being the same at 50 mg. each in the 2 groups and the means only slightly different (groups D and E). Thus, one may conclude that copper does not significantly change the reaction of the ovaries to pituitary implants when either the stimulus is minimal, as when one male rat pituitary gland is given, or when the stimulus is considerably above threshold.

9377 P

Experimental Alveolar Bone Atrophy Produced by Ascorbic Acid Deficiency and Its Relation to Pyorrhea Alveolaris.*

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Vitamin C (ascorbic acid) has been characterized by Wolbach and Howe[1] as necessary for the production of intercellular substances. Failure of connective tissue cells to form collagen matrices adequately accounts for the clinical signs and pathology of scurvy.

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[3] Emery, F. E., *et al.*, Proc. Soc. Exp. Biol. and Med., 1931, **29**, 42.

* Aided by a grant from the Milton Fund to the University Committee on Research in Dental Medicine.

[1] Wolbach, S. B., and Howe, P. R., *Arch. Path.*, 1926, **1**, 1.

Although loosening of the teeth of both man and animals in scurvy has been recognized,[2, 3] there has been little study reported of the pathological changes of the peridental tissue. The striking alterations in the tooth pulp and dentin have diverted attention from the peridental soft and calcified tissues.[4, 5] The influence of a chronic deficiency on these tissues has not been adequately described.

Pyorrhea is classified into two main types—(1) a local inflammatory process beginning at the gingival margin; (2) a systemic process characterized by diffuse atrophy of the alveolar bone.[6] Type II is recognized clinically by a loosening and wandering of the teeth, generalized rarefaction of the alveolar bone, and widening of the peridental membrane. Resorption of alveolar bone and cementum with loss of the collagenous fibers of the peridental membrane and depletion of the hematopoietic cells of the bone marrow are the essential microscopic findings. Pocket formation and suppuration are not characteristic of the early stages but may result from infection superimposed later in the disease process.[7]

Our findings in the peridental tissues of cases classified on a clinical basis as Type II pyorrhea corroborate those of Gottlieb.[6] Changes in the alveolar bone and peridental tissues are identical with those found in the peridental tissues in cases of unquestionable infantile scurvy[8] and with changes in animals maintained on diets deficient in ascorbic acid.

In the present study, a large number of guinea pigs have been kept for periods up to 6 months on a diet free of vitamin C, supplemented by measured amounts of ascorbic acid (0.3 to 5.0 mg. per diem), together with adequate controls. The head has been split in a sagittal plane, and X-rayed. Ground and decalcified sections in cross and in longitudinal planes, of both incisor and molar teeth and surrounding tissues, have been prepared.

The paradental tissues, in acute scurvy, are so weakened that the teeth may be easily extracted. Congestion of the gingiva is a constant finding, but pocket formation rarely occurs. X-ray and histological examination reveals a marked rarefaction of the alveolar bone and widening of the peridental membrane. These changes are

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[2] Westin, G., *Dent. Cosmos*, 1925, **67**, 868.

[3] Hess, A. F., *Scurvy, Past and Present,* Philadelphia, 1920, J. B. Lippincott Co.

[4] Hanke, M. T., *Diet and Dental Health,* Chicago, Ill., 1933, University of Chicago Press.

[5] Höjer, J. A., *Acta Paediat.*, 1924, Supp. 3: 8.

[6] Gottlieb, B. L., *J. Am. Dent. Assn.*, 1927, **14**, 2178.

[7] Kronfeld, R., *Histopathology of the Teeth,* Philadelphia, 1933, Lea and Febiger.

[8] Boyle, P. E., *J. Dent. Res.*, 1934, **24**, 172.

conspicuous over the labial enamel-covered part of the incisor teeth in the region anterior to the fulcrum of tooth movement. The failure of collagen-fiber formation which normally accompanies continuous eruption of the tooth results in weakening of the suspending apparatus. Ultimately the peridental fibers are unable to sustain the forces of mastication, the teeth become loosened, and atrophy of the bone results in the direction toward which pressure is exerted —(*i. e.,* toward the labial alveolar bone).

Similar changes take place at a slower rate in the tooth and peridental structures of animals on low ascorbic acid levels. The gross, X-ray, and histological deviation from normal depend upon the amount of ascorbic acid administered.

Thus in both acute and chronic ascorbic-acid deficiency, generalized rarefaction of the bone occurs. This rarefaction is a result of the inability of the osteoblastic cells to form bone matrix and is accompanied by a similar failure of the fibroblastic cells to form collagen fibers. The loosening and wandering of the teeth is an expression of the weakness of the bone and collagen-fiber suspending apparatus. The histological details of atrophy of the bony, fibrous, and hematopoietic tissues in the tooth and mandible are the classical changes of scurvy.[1, 9] Such atrophic changes likewise are the characteristic features of peridental disease of the diffuse alveolar bone atrophy type.

Ascorbic-acid determinations on the blood of a limited number of patients with peridental disease[10] by the use of a modification of a previously developed test[11] indicate a positive correlation between low ascorbic-acid blood levels and rarefaction of the alveolar bone not solely attributable to local inflammatory processes. This is consistent with the above evidence that suboptimal ascorbic-acid nutrition is the important factor in the production of the diffuse atrophy type of pyorrhea.

Summary. The essential characteristic of the diffuse atrophy type of pyorrhea *(pyorrhea alveolaris),* which is an inability of the peridental tissue to withstand functional stress, has been reproduced in guinea pigs maintained on diets deficient in ascorbic acid. The histological details in bone and connective tissue of these scorbutic animals are identical with those found in human peridental disease of this type.

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[9] Aschoff, L., and Koch, W., *Scorbut, Eine Pathologisch anatomische studie,* Jena, 1919, Gustav Fisher.

[10] Weisberger, D., and Bessey, O. A. Unpublished data.

[11] Bessey, O. A., and King, C. G., *J. Biol. Chem.,* 1933, **103**, 687.

9378 P

Effect of Adrenal Cortical Hormone on Reduction of Plasma Volume Resulting from Etherization.*

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Recent determinations with the blue dye T-1824 have shown that etherization of dogs causes a marked reduction (average 12%) in plasma volume.[1] Because the adrenal cortex appears to be concerned with the regulation of blood volume (Swingle, *et al.*,[2] Harrop, *et al.*[3]), it seemed of interest to study the effect of the administration of adrenal cortical hormone‡ on this fluid shift occurring under ether.

The changes in plasma volume were calculated from alterations in a dye disappearance curve established by spectrophotometric analysis of serum samples (Gregersen, *et al.*[4, 5]). Plasma volume changes were also calculated, with considerable agreement, from variations in the concentration of plasma proteins (measured with refractometer). Five intravenous injections of adrenal cortical extract, totalling not more than 30 cc. (3,000 dog units§) were made during each experiment; the first of these injections (usually 500 to 1000 dog units) was given prior to etherization, the remaining 4 at approximately 15-minute intervals during anesthesia. It has been shown that intravenous injections of adrenal cortical hormone must be repeated at frequent intervals to produce a prolonged effect (Thorn[6, 7]).

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* Aided by a grant (to Dr. M. I. Gregersen) from the Rockefeller Foundation.

†Rockefeller Fellow in Medicine.

[1] McAllister, F. F., *Proc. Am. Physiol. Soc.*, in press.

[2] Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., and Parkins, W. M., *Science*, 1933, **77**, 58.

[3] Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 1.

‡ The adrenal cortical extract used in these experiments was supplied by Dr. David Klein of the Wilson Laboratories, Chicago.

[4] Gregersen, M. I., Gibson, J. J., and Stead, E. A., *Proc. Am. Physiol. Soc.*, 1935, **113**, 54.

[5] Gregersen, M. I., to be published.

§ "Dog units" refers to an assay standardized on adrenalectomized dogs.

[6] Thorn, G. W., Garbutt, H. R., Hitchcock, F. A., and Hartman, F. A., *Endocrinology*, 1937, **21**, 213.

[7] Thorn, G. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 361.

In 5 experiments, repeated intravenous injections of adrenal cortical extract, in every case, modified the reduction in plasma volume which is consistently produced by ether anesthesia. When a delay of 4 or more minutes occurred between the initial dose of extract and the application of ether, the plasma volume first decreased and then with successive injections quickly returned to normal. When anesthesia was induced immediately after the first dose of extract, the initial reduction did not occur and the normal plasma volume was maintained. The results of a series of experiments on one of the dogs are presented in Table I.

TABLE I.
Effect of Adrenal Cortical Hormone on Plasma Volume During Etherization.

Dog No.	Date	Weight	Decrease in Plasma Volume during Ether Anesthesia		Remarks
		kg.	cc.	%	
2M	Dec. 10	14.9	90	10.0	Control
2M	Jan. 4	15.4	95	10.4	”
2M	Jan. 26	16.4	90	9.7	”
2M	Mar. 17	15.8	0	0	Adrenal Cortical Hormone*

* A total of 2,800 dog units was administered intravenously in 5 doses.

Four of the animals showed, on recovery, either a maintenance of their normal plasma volume or a rise above it. The fifth animal suffered a gradual reduction in plasma volume beginning near the end of anesthesia and continuing for 2 hours thereafter. Such reactions may normally occur after etherization and are probably not due to the cortical extract.

Determinations of plasma sodium and chloride were made on blood samples drawn immediately preceding, during and 3 hours following anesthesia. No significant difference was noted in the concentration of these electrolytes in the serum of control animals as compared with those treated with the hormone.

Conclusion. Preliminary experiments demonstrate that repeated intravenous injections of adrenal cortical extract may act to preserve the normal plasma volume under ether anesthesia.

9379

Goitrogenic Effect of Dietary Fat.

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Marine[1] believes that thyroid activity may be more necessary for oxidation of fats than of carbohydrates. Harington[2] suggests that fats, especially unsaturated ones, may interfere with iodine absorption by the thyroid. Chidester[3-6] states that unsaturated fats absorb iodine from the body. Few positive experiments have been reported to support these beliefs. McCarrison[7] found that diets excessively rich in proteins and fats caused thyroid enlargement in pigeons. The Mellanbys[8] noticed that puppies receiving cod-liver oil as their only fat had small, normal thyroids, but with other fats there was always some hyperplasia, which was intensified when the amount of dietary fat was increased. Levine and Remington,[9] in a comprehensive review, do not refer to the goitrogenic action of fats.

White rats, weighing about 50 gm., were kept in cages which prohibited coprophagy. The diets are given in Table I. Olive and cocoanut oils were chosen so that the effects of relatively saturated and unsaturated fats might be compared, olive oil being a typical unsaturated fat, and cocoanut oil, saturated. The salt was the usual Osborne and Mendel[10] mixture, except that the potassium iodide was decreased. The salt mixture of diets A and B contained 13.5 mg. of potassium iodide per 500 gm., as compared with 4.6 mg. in diets C and D. The 2 diets containing olive oil differed only in the content of iodine. Levine, Remington, and von Kolnitz[11] have found that 1-2 gammas of iodine per day are sufficient to prevent goiter in the rat. The iodine in our salt mixtures supplied an excess well over the minimum requirement. (Table II.) The iodine content of the other dietary constituents was not determined. Vitamins were supplied by brewer's yeast, viosterol, and caritol.* At the end of 50-60 days, the rats were killed and the thyroids were removed, weighed immediately, and then preserved in formalin for histological study.

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[1] Marine, D., *Physiol. Rev.*, 1922, **2**, 521.

[2] Harington, C. R., *Lancet*, 1935, **228**, 1199.

[3] Chidester, F. E., *Med. World*, 1935, **53**, 649.

[4] Chidester, F. E., *Am. Med.*, 1934, **40**, 162.

[5] Chidester, F. E., *Science*, 1932, **75**, 286.

[6] Chidester, F. E., *Med. World*, 1935, **53**, 167.

[7] McCarrison, R., *Indian J. Med. Research*, 1919, **7**, 633.

[8] Mellanby, E., and Mellanby, M. J., *J. Physiol.*, 1921, **55**, Proc. Physiol. Soc., vii.

[9] Levine, H., and Remington, R. E., *J. Chem. Ed.*, 1933, **10**, 649.

[10] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913, **15**, 317.

[11] Levine, H., Remington, R. E., and von Kolnitz, H., *J. Nutrition*, 1933, **6**, 347.

TABLE I.

Diet	Protein %	Fat %	Carbohydrate %	Salt Mixture %
A	Casein 18	Olive oil 28	Cornstarch 50	4
B	" "	Cocoanut oil "	" "	4
C	" "	Olive oil "	" "	4
D	" "	None	" 78	4

Histological examination† revealed no pathology, and showed that the glands were essentially alike regardless of the sex of the rat or the type or amount of fat fed. They showed a cubical acinar epithelium of normal appearance and an abundant storage of colloid, probably near the upper limit of the normal.

TABLE II.

Diet	No. of rats	Aver. daily gain gm.	Daily iodine intake* gamma	Thyroid wt., mg.	Thyroid per 100 gm. body wt., mg.
A	5	2.02	6.55	16.3	10.3
		(1.77-2.17)†	(5.98-6.60)	(11.1-30.9)	(7.3-19.5)
B	4	1.72	6.90	15.1	10.5
		(1.42-1.87)	(6.60-7.22)	(13.7-18.0)	(9.7-12.8)
C	5	2.27	2.43	20.6	10.6
		(1.78-2.62)	(2.06-2.68)	(17.0-24.0)	(9.8-12.4)
D	5	2.19	3.79	16.7	8.6
		(1.73-2.87)	(3.51-4.54)	(13.5-19.0)	(7.0-10.4)

*This comprises iodine content of salt mixture only.

†The figures in parentheses represent the extremes of individual variations in each group.

Table II shows that the degree of saturation of a fat is not a factor in thyroid enlargement. A comparison of the thyroids of animals fed a diet of low iodine and high fat content (Diet C) with those of animals fed a diet low in its iodine content but containing no added fat (Diet D), indicates a slightly increased weight of the thyroids of Group C (an increase of approximately 20%). However, this is hardly significant, since Levine, Remington, and von

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* The authors are indebted to the S.M.A. Corporation, Cleveland, Ohio, for the supply of caritol.

† The authors are indebted to Dr. Carl V. Weller of the Department of Pathology of the Medical School of the University of Michigan, for the histological examination of the thyroid glands.

Kolnitz[12] have shown that rat thyroids 400-1000% above the normal weight can be produced by a goitrogenic diet.

Since this work was completed, Remington has reported, at the April, 1937, meeting of the American Chemical Society, that neither saturated nor unsaturated fats, up to a level of 13% of the diet, have any measurable effect upon the size, dry matter, or iodine content of the thyroid gland. Our results are in accordance with his. Under the conditions of our experiments, neither the type nor the amount of fat in the diet had any demonstrable influence upon the weight or histology of the thyroid.

9380

Observations on the L-Organism of Klieneberger.

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The *streptobacillus moniliformis,* under various names, has been reported to occur in rats in pulmonary disease,[1,2,3] otitis media,[4] and as inhabitant of the nasopharynx;[5] in mice, as an agent of systemic disease;[5,6,7] in humans, in fevers following the bite of rats or other small animals,[8-11] or as an epidemic[12] or isolated[13] infection of variable transmission. A similar organism has been observed by Smith[14] in pneumonia of calves. All organisms described in the above reports are characterized morphologically by huge bulbous or club-like swellings, which appear early in a developing culture, and later make up a large part of it. These swollen forms gained great theoretical importance when Klieneberger[15] observed that they really represent an organism separate from the bacillus, which could be cultivated and grown alone, showing no reversion to bacillary forms in the 2 years of observation. Furthermore, the properties of this organism (designated "L" by Klieneberger) differ in many respects from those of bacteria and fungi, and resemble the curious organisms of *pleuropneumonia bovis* and of agalactia. The L-organism being very soft and fragile, its real form is difficult to ascertain. It is known, however, to be very pleomorphic, exhibiting large disc-like forms, small filter-passing granules, and filaments of greatly varying size. Thus we have an entirely new type of observation before us: The cultures of a bacillus causing disease both in animals and in man appear to consist of 2 organisms, one of which shows properties associating it with organisms usually classed with the filtrable viruses. Recently Klieneberger[3] has found that the pleuropneumonia-like organism is frequently found alone, without the bacillus, in the lung-abscesses of rats. In contrast, however, all observations indicate that the streptobacillus cannot be freed of the characteristic swollen forms of the L-organism.

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[12] Levine, H., Remington, R. E., and von Kolnitz, H., *J. Nutrition*, 1933, **6**, 325.

[1] Tunnicliff, R., *J. Inf. Dis.*, 1916, **19**, 767.

[2] Jones, F. S., *J. Exp. Med.*, 1922, **35**, 361.

[3] Klieneberger, E., and Steabben, D. B., *J. Hyg.*, 1937, **37**, 143.

[4] Nelson, J. B., *J. Inf. Dis.*, 1930, **46**, 64.

[5] Strangeways, W. I., *J. Path. and Bact.*, 1933, **37**, 45.

[6] Levaditi, C., Selbie, R. F., and Schoen, R., *Ann. Inst. Pasteur*, 1932, **48**, 308.

[7] Mackie, T. J., Van Rooyen, C. E., and Gilroy, E., *Br. J. Exp. Path.*, 1933, **14**, 132.

[8] Schottmüller, H., *Derm. Woch.*, 1914, **58** (Supp.), 77.

[9] Blake, F. G., *J. Exp. Med.*, 1916, **23**, 39.

[10] Dick, G. F., and Tunnicliff, R., *J. Inf. Dis.*, 1917, **23**, 183.

[11] Scharles, F. H., and Seastone, C. V., *New Eng. J. Med.*, 1934, **211**, 711.

[12] Parker, F., Jr., and Hudson, N. P., *Am. J. Path.*, 1926, **2**, 351.

[13] Levaditi, C., Nicolau, S., and Poincloux, P., *Compt. Rend. Acad. Sci.*, 1925, **130**, 1188.

[14] Smith, T., *J. Exp. Med.*, 1918, **28**, 333.

In the course of some studies on rats undertaken in the Anaphylaxis Laboratory, the opportunity arose to make some observations upon the symbiont present in cultures of *Streptobacillus moniliformis.* These observations confirm Klieneberger's work and extend the knowledge of the pathogenicity of the L-organism. The middle ear of a rat showing typical "twisting" was opened, and the small amount of pus found therein was planted on a medium similar to that recommended by Klieneberger[15] (centrifuged boiled blood agar added to an equal volume of horse serum). In 2 to 3 days about half a dozen typical *Streptobacillus moniliformis* colonies developed on the plate. The remainder of the plate was densely covered with tiny colonies corresponding to those of Klieneberger's L-organism. Impression-preparations of the bacillary colonies always showed the bacteria clearly, but bacterial forms were never found in the first or subsequent subcultures of the L-organism. These have been maintained pure and viable by frequent transfer for 5 months to date (May, 1937), and have been submitted to several animal passages. The L-organism has preserved its peculiar character and has not reverted to a bacterial form.

Another strain of the organism was obtained, in association with various bacillary forms, from a gastric abscess in a rat. We examined also about 12 large and small abscesses in rats' lungs, and found repeatedly in the sticky pus the short filamentous forms seen first by Tunnicliff,[1] and identified later with the L-organism by Klieneberger.[3] But neither in solid nor liquid media, nor by mouse-inoculation could we obtain any growth of these organisms, although in 3 cases the streptobacillus grew out.

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[15] Klieneberger, E., *J. Path. and Bact.*, 1935, **40**, 93.

A strain of particular interest because of its source was obtained from an excised suppurating Bartholin's gland from a human patient. On ascitic agar the pus yielded a very dense growth of L-forms, without any accompanying bacteria. The patient was a laboratory worker coming in frequent contact with rats. The clinical description of this case will be reported elsewhere.[16]

Klieneberger published no data concerning the pathogenicity of her cultures. Our cultures possessed a marked pathogenicity for white mice. We injected white mice subcutaneously and intraabdominally with small pieces of agar containing a dense pure growth of tiny L-colonies, ground and suspended in saline. The mice died in from one to 10 days. Animals dying in approximately one to 3 days showed no pathologic changes except a very slight pleural exudate. Mice dying later showed abundant clear pleural exudate, and in some animals a thick subcutaneous edema developed over the whole ventral surface of the body. In animals injected intraäbdominally, more or less peritonitis and peritoneal exudate were also present, and some of these animals also developed subcutaneous edema. From the pleural, peritoneal and subcutaneous exudates the L-organism grew abundantly in pure culture, but no organisms were demonstrable in the exudate by direct microscopic examination, using either the dark field, or various staining methods. The heart's blood was sterile in all cases except one, dying within 24 hours, which yielded a few L-colonies. It is interesting to note that the disease produced by the pure L-organism differs in some respects from that ascribed to the streptobacillus in previous observations.[5, 6, 7, 17]

The pathogenicity of the L-organism for mice is not uniform. Our first strain when first tested killed 2 mice in 4 days. After 2 animal-passages death occurred only after 5, 6 and 10 days. Some fully grown white mice, previously employed in pneumococcal typing, showed no symptoms following injection of L-organism suspensions. The mice dying in 1 to 3 days, on the other hand, were half-grown animals raised in the laboratory. Twelve mice injected subcutaneously with large amounts of pus from the lung-abscesses of rats developed no signs of any infection. Thus it is possible that both the condition of the strain and of the animal influences the pathogenic action.

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[16] Dienes, L., and Parsons, L., to be published.

[17] Van Rooyen, C. E., *J. Path. and Bact.*, 1936, **43**, 455.

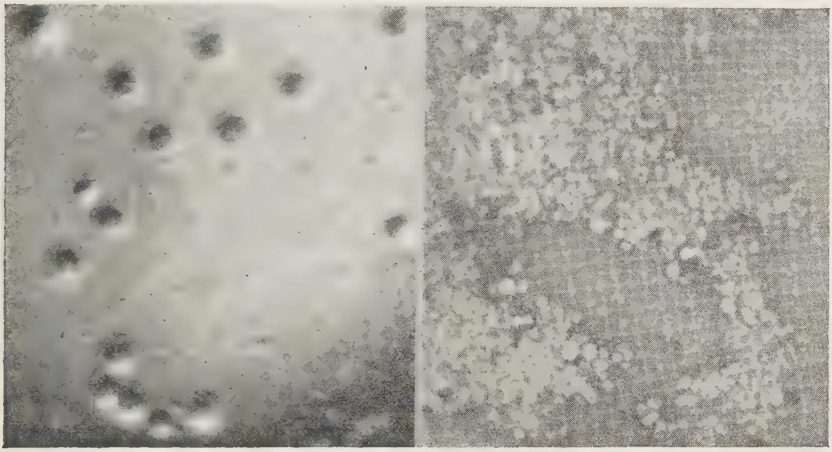

FIG. 1. FIG. 2.

Fig. 1 shows the colonies of L-organism. Original plate from pus found in middle ear of a rat. (About 50 ×.)

Fig. 2, impression preparation from similar colonies; dark background with mercurochrome (1000 ×).

Our observations concerning the morphology of the L-organism confirm those of Klieneberger. We have never seen bacillary or coccal forms in the L-type cultures. The L-form is distinguished morphologically from the accustomed forms of bacteria and fungi by its great fragility and pleomorphism. Our cultures in most instances seemed to consist only of disc-like forms of different sizes. But sometimes, both in our strains and in one furnished by Klieneberger, filaments were present in abundance. The connection between the streptobacillus and the L-organism is entirely obscure. The morphologic development of streptobacillary colonies rather suggests that the L-form, if it is not a derivative of the bacillus, is either a parasite or an internal symbiont thereon, and not simply an admixture to the culture. The large, swollen forms seem to arise by a direct transformation of the bacteria and at first there is no evidence for their independent multiplication. Their subsequent independent growth in the culture, however, shows that they are not the degenerative products of the bacteria, as suggested by Van Rooyen,[17] but are a definite viable form of organism.

Summary. In confirmation of Klieneberger's observation, the L-organism was cultivated from rats. It was also cultivated from a human suppurating lesion. The strains were morphologically and culturally similar to those of Klieneberger, and maintained their character during 2 to 5 months' observation. The L-organism exerts a definite pathogenic action on mice.

9381 P

Effects of Minute Amounts of Lead in the Diet of the Dog.

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The observation that 100 mg. of lead per kilo of diet did not cause any apparent change in the growth, blood and reproductive performance of the rat,[1] led to the extension of the study to another species, namely, the dog. Two litters of 3 puppies each were divided into 3 groups, so that one dog from each litter was in each group, and fed a diet containing 41.2% casein, 29.4% sucrose, 18.3% lard, 7.2% butter, 2.6% bone ash and 1.3% of Cowgill-Karr salt mixture.[2] Seventy grams of dried yeast and 10 gm. of cod liver oil were added to each kilo of diet. Measured amounts of lead acetate were mixed with the salts so that the increment of lead for each group was 0, 25 and 100 mg. of lead per kilo of diet, respectively. The puppies grew well on this ration. Litter I, born of a 12-kilo mongrel, gained an approximate average of 1500 gm. per month and litter II, born of a fox terrier, gained about 700 gm. per month. The added lead in the diets of groups 2 and 3 did not seem to affect the comparative growth of the dogs. One dog in group 3 was a bit smaller than its litter mates, but this animal was quite plump and its decreased size probably was due to the fact that it had more of a smaller breed in its genetic constitution.

Weekly examinations were made of the blood of each dog for hemoglobin content, erythrocyte count and basophilic granulations (stippling) when stained with Wright's blood stain.

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* Lead Research Fellow.

[1] Horwitt, M. K., and Cowgill, G. R. In process of publication, Reported at Meetings of the American Society of Biological Chemists at Memphis, April, 1937.

[2] Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725.

After 225 days on the experimental diet, the hemoglobin contents of the blood of the dogs in gm. % were 13.7 and 13.3 for group 1, 13.7 and 13.3 for group 2, and 12.3 and 12.2 for group 3. The erythrocyte count expressed in millions of cells per cubic mm. of blood was 6.30 and 6.10 for group 1, 6.60 and 6.80 for group 2, and 8.91 and 7.59 for group 3. No stippled cells were found until after 2 months on the experimental diet when a considerable amount of basophilic stippling became apparent in both dogs from group 3 (102 mg. per kilo). This apparent disagreement with the suggestion by Aub, *et al.*,[3] that dogs' blood does not stipple may be explained by the fact that we were dealing with puppies which because of their youth, were probably more susceptible to lead. No stippled cells were found in the blood of the dogs in groups 1 and 2.

The observation that the comparative increase in erythrocytes in group 3 was not associated with a corresponding increase in the hemoglobin content of the blood indicates that the individual erythrocytes were deficient in hemoglobin.

After 7 months on the experimental diet one of the dogs in group 3 became ill quite suddenly with bloody diarrhea and severe colic contractions. The dog was sacrificed, a rib removed for lead analysis, and representative sections of the brain, lung, kidney, and intestines saved for pathological analysis. A few days later the 2 dogs on the control diet developed an upper respiratory infection. At this time all of the remaining 5 dogs were sacrificed and sections of their organs saved for analysis. Table I presents the results of analysis of samples of blood taken from the dogs prior to death together with the results of the lead analysis of the ribs.[4]

The animal from group 3 which became ill had 230 mg. of lead per kilo of bone and had ingested the highest amount of lead. This was the only animal whose tissues on examination showed any pathological changes that might be interpreted as lead poisoning and even in this animal the changes were doubtful.

TABLE I.
Comparison of Lead Content of Blood and Bones with Amounts of Lead Ingested.

	Group 1 2 mg./kilo		Group 2 27 mg./kilo		Group 3 102 mg./kilo	
Mg. lead per kilo of blood	.40	.36	.62	.45	.67	.72
Mg. lead per kilo of bone	6.1	13.5	36.7	69.8	230.	198.
Gm. lead ingested	.094	.066	1.269	.756	4.59	2.86
Age of dogs in days	232.	292.	232.	293.	226.	295.
Final wt., kg.	12.5	5.0	12.8	5.5	9.3	5.0

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[3] Aub, C. A., Fairhall, T., Minot, A. S., and Reznikoff, P., *Medicine Monographs*, 1926, **7**, 139.

[4] Description of authors' method to appear in *J. Biol. Chem.*, July, 1937.

Except for the appearance of occasional stippled erythrocytes, the other dog in group 3 was apparently normal. A neurological examination† failed to show anything abnormal in any of the dogs. X-ray examination of the bones of all the animals was made during various stages of growth in an effort to detect lead deposition, but it was not possible to notice any significant differences between the X-ray photographs of the dogs.

9382

Effect of Vitamin E Deficient and Muscular Dystrophy-Producing Diet on Metabolism of Guinea Pigs.

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It has been found[1, 2] that excised skeletal muscle from animals exhibiting nutritional muscular dystrophy shows an increased consumption of oxygen. The thyroid glands from rats deprived of vitamin E are markedly hypoplastic.[3] Endocrine gland disturbances have frequently been observed in clinical muscular dystrophy.

This report is concerned with the results of metabolic rate determinations made upon guinea pigs kept on diet 13 of Goettsch and Pappenheimer[4] supplemented by 8 cc. of fresh tomato juice daily. This diet is deficient in vitamin E and also allows the development of muscular dystrophy. Ten young guinea pigs (5 male and 5 female) served as experimental animals. Control guinea pigs of the same age were selected from the same stock and kept upon a diet of grains, alfalfa and lettuce. Measurements of oxygen consumption were made at a room temperature of 28°C. in a multiple closed circuit apparatus (Benedict).[5] The determinations were made at approximately one week intervals. A total of 116 determinations were made on the experimental animals and 55 on the control group. Food was withheld for 24 hours before each experiment. The presence of the typical lesions of experimental muscular dystrophy was established by examination of the skeletal muscle removed at necropsy.

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† We are indebted to Dr. James C. Fox, Jr., for making this examination.

[1] Victor, J., *Am. J. Physiol.*, 1934, **108**, 229.

[2] Madsen, L. L., *J. Nutrition*, 1936, **11**, 471.

[3] Singer, E., *J. Physiol.*, 1936, **87**, 287.

[4] Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

[5] Benedict, F. G., *J. Nutrition*, 1930, **3**, 161.

TABLE I.

		Body wt. in gm.			Cc. O_2 used per M^2 of Surface Area per Hr.		
Exper. Condition	Days on Diet	Initial	Maximum	Terminal	Initial	Final	Aver. all tests
Controls	118	258	511	495			6278
Males, Diet 13	69	342	422	386	6897	6574	6314
Females, Diet 13	102	278	412	390	6193	6645	6433

The data (Table I) show that no significant difference exists between the metabolic rates, expressed as cc. of O_2 used per hour per square meter of surface area, of control and dystrophy animals. Furthermore, the rates of oxygen utilization by the animals in the early days of the experiments were essentially the same as that found in the terminal experiments on the same animals.

If an endocrine disturbance existed in such animals, reared under the above conditions, it was not of such a nature or degree as to be reflected in their rates of oxygen utilization. These observations on the intact animal offer no explanation as to the cause of the increased oxygen consumption by excised skeletal muscle from animals under similar dietary conditions.

Summary. Guinea pigs reared on a vitamin E deficient diet and exhibiting typical experimental muscular dystrophy show normal metabolic rates as determined by oxygen consumption measurements.

9383 P

Recovery of Influenza Virus from Chicago Epidemic.

J. Marion Clampit and F. B. Gordon. (Introduced by G. M. Dack.)

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In the original isolation of the virus of human influenza, ferrets were used as the experimental animal.[1] Later it was found that after a few passages in ferrets the virus became pathogenic for young white mice.[2] Since some of the strains required only one or 2 ferret passages before becoming pathogenic for mice, numerous attempts have been made to infect mice by means of material taken directly from patients. Francis and Magill[3] have recently succeeded in infecting mice with patients' nasal washings concentrated by ultracentrifugation. The disease in mice is characterized by loss of appetite, respiratory difficulty, malaise, and death usually by the fifth day. Lung consolidation with a mononuclear cell infiltration is the essential feature of the autopsy findings.

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[1] Smith, W., Andrews, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.

[2] Andrews, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2**, 859.

[3] Francis, T., and Magill, T. P., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 132.

Using direct mouse inoculation we were able to recover a filterable infectious agent, identified as human influenza virus, from each of 9 specimens of sputum and nasal washings. These were obtained from 7 patients during a small epidemic of influenza in Chicago early in December, 1936. The material was collected 12 to 72 hours after onset, taken to the laboratory, ground with powdered glass and emulsified with veal infusion broth. After light centrifugation the supernatant material was used for inoculation of mice, according to the method originated by Shope[4] in his work with swine influenza. This consists of placing the nose of the anesthetized mouse under the surface of the inoculum for several breaths. Inoculations of passage mice were made in the same manner by means of a heavy emulsion of lung tissue. Four to 6 young mice were used for each of the original inoculations and 3 for each subsequent passage. The mice were sacrificed at 5-day intervals and the lungs examined for gross lesions. For the first passages pooled material from all of the lungs were used for inoculum. Later only consolidated portions were used. The strains varied in producing initial consolidation; the earliest appeared in the 3rd passage, the latest on the 8th. Five to 13 passages were necessary before consistent complete consolidation was produced, usually with death by the fifth day. After becoming adapted, all 9 strains produced identical results in mice. The gross and microscopic pathology were similar to that of lungs infected with a known influenza virus.

Evidence that this condition was caused by a filterable agent consists of the following: (1) Aerobic and anaerobic cultures of the lungs were usually negative after the first passage. A gram negative rod was cultured inconstantly. (2) Eight of the 9 strains have been filtered through a Berkefeld N candle, the filtrate producing consolidation in all cases. No aerobic or anaerobic bacteria could be cultured from the filtrate. (3) Two of the strains have been stored in 50% glycerin for 3 months without noticeable loss in virulence.

A control series was run exactly as in the original isolations except that an emulsion of a normal mouse lung was used as the first inoculum. No consolidation has occurred in 9 passages. Intraperitoneal injection of mice with an infectious lung emulsion had no effect, which indicates that the virus of ectromelia, an infectious disease of mice, is not present.

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[4] Shope, R. E., *J. Exp. Med.*, 1935, **62**, 561.

The circumstances of isolation and the pathology produced suggested that we were dealing with human influenza virus. Positive identification was accomplished by means of cross neutralization tests with a known human influenza virus, the PR8 strain. Antisera were obtained by intraperitoneal inoculation of the various strains into rabbits. For the test equal parts of a 2% emulsion of consolidated lungs and dilutions of antisera were mixed and incubated for 30 minutes at 37°C. Three mice were inoculated intranasally with each mixture. On the seventh day all survivors were sacrificed and the lung consolidation recorded. The results of the test are given in Table I. From these results our strains tested to date seem to be identical serologically with the PR8 strain.

TABLE I.
Results of Cross Neutralization Tests with Strains of Influenza Virus from Chicago Epidemic.

Antisera		Viruses		
Strain	Dilution	PR8	W2	Pe
PR8	1/1	0 0 0	0 0 0	0 0 0
	1/5	0 0 0	0 0 0	0 0 0
	1/25	0 0 0	1 2 2	2 2 2
	1/125	0 4 4	2 3 3	3 4 4
W2*	1/1	0 0 0	0 0 0	
	1/5	0 0 0	0 0 0	
	1/25	0 1 1	1 1 1	
	1/125	2 4 4	3 4 4	
Pe*	1/1	0 0 0		0 0 0
	1/5	0 0 0		0 0 0
	1/25	0 1 4		0 1 2
	1/125	4 4 4		3 4 4
V.I.B.		4 4 4	4 4 4	4 4 4
N.R.S.		4 4 4	4 4 4	4 4 4

Each number represents one mouse and refers to the degree of lung consolidation. 0 = no consolidation. 4 = total consolidation.
V.I.B. = veal infusion broth. N.R.S. = normal rabbit serum.
*Strains isolated from Chicago epidemic.

Summary. A filterable agent has been recovered from sputum and nasal washings of patients in the acute stage of influenza. This was accomplished by direct intranasal inoculation of mice. Evidence has been produced which indicates that the strains are human influenza virus.

9384

The Metabolism of Pathogenic Yeasts.*

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Frequent reference is made in the literature[1] to the fermentation of sugars by pathogenic yeast-like organisms. Cultures of such organisms in sugar-rich media may evolve much CO_2 and have a "beery" odor. Quantitative data as to the extent of the alcoholic fermentation are not, as far as we are aware, available.

The organisms were obtained from various sources, as indicated in Table I. We are particularly indebted to Dr. H. C. Hesseltine for identification of organisms 4 to 14 inclusive. Six of these were identified as *Monilia albicans* and 5 as *Monilia candida.*

The medium contained one percent of peptone (Difco), 0.3% of meat extract, 0.2% of KH_2PO_4, traces of $CaCl_2$ and $MgSO_4$, enough NaOH to bring the reaction to pH 7.6, and 5% of glucose. The glucose, in 20% solution, was autoclaved separately. Four liters of this medium were prepared at one time and mixed thoroughly with the sugar immediately after autoclaving. Exactly 200 cc. were then pipetted into tall 8 oz. dispensing bottles. With the exception of the brewers' yeast, organism 1, which was incubated at room-temperature, the cultures were kept 48 hours at 37.5°C. Exactly 20 cc. of N H_2SO_4 were then added to stop metabolic activities. The bottles were kept in the refrigerator until analyzed. The following analytical methods were used: Glucose, Shaffer-Somogyi;[2] ethyl alcohol, Friedemann and Ritchie;[3] lactic acid, Friedemann and Graeser.[4] The total acids were determined by titration of 25 cc. samples with 0.1 N NaOH after 15 minutes of rapid aëration with CO_2-free air. The gases were qualitatively examined for CO_2 and hydrogen.

The results shown in the table represent the decrease in sugar and

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* This study was supported by a grant from the Bartlett Memorial Fund, University of Chicago.

[1] Castellani, A., *Arch. Derm. Syphilis,* 1928, **17**, 61; Zinsser, H., and Bayne-Jones, S., *Textbook of Bacteriology,* D. Appleton-Century Co., 7th edition (1934).

[2] Shaffer, P. A., and Hartmann, A. F., *Biol. Chem.,* 1920-21, **45**, 365; Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry,* Baltimore, 1932, **2**, 465.

[3] Friedemann, T. E., and Ritchie, E. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 451.

[4] Friedemann, T. E., and Graeser, J. B., *J. Biol. Chem.,* 1933, **100**, 292.

TABLE I.

No.	Organism	Glucose used mM. per l.	Ethyl alcohol mM. per l.	Yield of alcohol % of theory	Total acids cc. N per l.	Source
1.	Saccharomyces, brewers' yeast	90	158	88	4.5	Anhäuser-Busch Brewing Co.
2.	" bread yeast	270	524	97	9.4	Fleischmann Co. culture No. 40
3.	" " "	256	491	96	7.0	" " " " 135
4.	Monilia	124	229	92	5.4	Vaginal mycosis*
5.	"	115	226	98	4.7	" " *
6.	"	148	275	93	3.6	" " *
7.	"	121	206	85	5.6	" " *
8.	"	244	423	87	14.6	" " *
9.	"	127	225	89	3.4	" " *
10.	"	109	200	92	4.0	" " *
11.	"	132	118	45	3.6	" " *
12.	"	102	159	78	3.4	" " *
13.	"	253	493	97	7.4	Bronchiectasis†
14.	"	128	230	90	5.4	" †
15.	" *albicans* No. 1886	129	236	92	6.1	Oral thrush‡
16.	*Cryptococcus hominis* No. 2396	191	350	92	9.5	White hairy tongue‡

*Cultures supplied by Dr. H. C. Hesseltine.

†From two cases of Dr. Harry L. Huber.

‡From Dr. Fred D. Weidman, Philadelphia.

increase in alcohol and total acidity as compared with the uninoculated acidified medium.

No increase whatsoever of lactic acid was noted in any of the cultures. All of the organisms produced acids, but the quantity was small and variable. From 4.5 to 9.4 cc. *N* acid with an average of 7.0 cc. was produced by the non-pathogenic yeasts. The 12 cultures of monilia produced from 3.4 to 14.6 cc. *N* acid, with an average of 5.6 cc. The chief products of metabolism were CO_2 and ethyl alcohol. The average yield of alcohol from the 3 non-pathogenic yeasts was 94%. Excluding the results from organism 11, the monilia yielded 90% of alcohol. *Cryptococcus hominis* yielded 92% of alcohol.

Conclusions. The principal products of 13 pathogenic yeasts (12 monilia and *Cryptococcus hominis*) in a buffered peptone-meat-extract medium, with 5% glucose, were ethyl alcohol and CO_2. The yield of metabolic products was identical with that from 3 non-pathogenic yeasts.

9385 P

I. Quantitative Measurement of Coproporphyrin and Total Coproporphyrin I Excretion in Normals.

K. DOBRINER, W. H. STRAIN AND S. A. LOCALIO. (Introduced by C. P. Rhoads.)

From the Department of Medicine, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

Coproporphyrin I is excreted in the urine and feces under normal and most pathological circumstances.[1, 2, 3] In order to determine the relations between normal and abnormal pigment construction and destruction it has been necessary to develop an adequate method for the exact quantitative separation and measurement of porphyrins. Urine and feces were collected for one- to 3-day periods and kept in the dark. After careful mixing aliquot portions were analyzed. Not less than 50 gamma of coproporphyrin should be available for measurement; normally this amount is present in about half the daily amount of urine and about one-quarter of the daily feces. The

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[1] Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

[2] Dobriner, K., *J. Biol. Chem.*, in press.

[3] Watson, C. T., *J. Clin. Invest.*, 1935, **14**, 110; 1936, **15**, 327.

remaining urine and feces were extracted for determination of melting points.

The reaction of the aliquot was made negative to Congo red by 10% sodium acetate. One-third of the volume of glacial acetic acid was added and the mixture shaken 3 times with 3 volumes of ether. The combined ether extracts were washed twice with dilute sodium acetate solution and the total porphyrin extracted quantitatively with 5% HCl. With sodium acetate and acetic acid the porphyrin was driven again into ether and extracted repeatedly and quantitatively from the ether after washing with dilute sodium acetate by equal volumes of 0.5% HCl. The 0.5% HCl was diluted to 0.2%, shaken repeatedly with alcohol-free chloroform until the chloroform was colorless. In the 0.2% solution only coproporphyrin is present. This is driven quantitatively into ether by means of sodium acetate and acetic acid. The ether was washed twice, extracted 3 times with small quantities of 5% HCl. These extracts were combined and brought to volume. A volume was chosen which gave a concentration of porphyrin about the same as the standard solution.

For the standard solution a 1.0 mg. % coproporphyrin I solution in 5% HCl was used. For the quantitative estimation a photoelectric colorimeter was employed (Goudsmit and Summerson[4]). Since traces of pigment are occasionally present which can be removed only with loss of porphyrin a combination filter from Corning Glass No. 349 and No. 429 was employed, allowing light 5320 to 6000 Ångstroms. Calculations may be made according to the usual methods of colorimetry. Check determinations are in agreement provided the extraction methods are uniform.

Quantitative determinations of urinary and fecal porphyrins were made in 5 normal adult patients over 9-day periods. The hematologic pictures and the amounts of urobilin excreted were within the normal ranges, indicating that the construction and destruction of respiratory pigments were in equilibrium. The daily excretions of coproporphyrin I varied moderately. The daily average excretions of coproporphyrin I are listed in Table I. The average of at least 6 consecutive daily determinations can be accepted as significant. The average values for the 5 adult cases studied fall within the same range, with a mean deviation of ±10%, whereas in the case of a child the values were somewhat lower.

Type I porphyrins cannot be derived by degradation of any of the known respiratory pigments, which are all Type III compounds.

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[4] Goudsmit, A., and Summerson, W. H., *J. Biol. Chem.*, 1935, **111**, 421.

TABLE I.
Quantitative Coproporphyrin I Excretion in Gammas in Average Daily Values for 9-day Period.

Case No.	Age	Sex	R.B.C. in millions	Hgb., gm. per 100 cc.	Coproporphyrin in gammas Urinary	Fecal	Total
1	12	F	4.5	15	35	191	226
4	21	M	4.6	13	87	231	318
2	25	M	4.5	15	123	205	328
5	33	M	4.59	14.4	102	274	376
3	35	M	5.08	15.9	121	241	362
6	72	M	4.3	14.8	64	242	306

They must be formed by synthesis of simple pyrrol complexes.[5] The simultaneous construction of Type I and Type III porphyrins, a phenomenon described in humans,[5] dogs,[6] and yeasts,[5] is called the dualism of the porphyrins.

Experimental observations as well as theoretical considerations suggest the following working hypothesis. *In vitro* porphyrins are formed by combination of substituted pyrrols to suitably constructed pyrromethenes. Two pyrromethenes are then condensed to porphyrins and mixtures of isomeric compounds are obtained. *In vivo* by enzymatic action or otherwise porphyrin synthesis is directed to the formation of large amounts of Type III porphyrins and a small amount of Type I porphyrin. Physiologically there is a direct proportion between the construction of Type III and Type I porphyrins. Simple increased or decreased Type III porphyrin construction associated with hematopoietic activity leads to a proportionally increased or decreased Type I porphyrin construction and excretion. Coproporphyrin-I excretion may, therefore, be an index of hematopoietic activity. In certain pathological states this fixed relation is disturbed so that a disproportionate Type-I construction occurs.[7] Finally, faulty construction or utilization of Type III compounds may lead to the production of Type III porphyrins.

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[5] Fischer, H., *Verhandl. deutsch. Ges. inn. Med.*, 1935, **45**, 7; *Handbuch der normalen u. pathologischen Physiologie*, Berlin, 1928, **6**, 164; 1932, **18**, 148.

[6] Dobriner, K., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 757.

[7] Dobriner, K., Localio, S. A., and Strain, W. H., *J. Biol. Chem.*, 1936, **114**, XXVI.

9386 P

II. Coproporphyrin I Metabolism and Hematopoietic Activity.

K. Dobriner, W. H. Strain, S. A. Localio, H. Keutmann and D. I. Stephens. (Introduced by C. P. Rhoads.)

From the Department of Medicine, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

In a previous communication concerning the porphyrin excretion in normal individuals[1] we have briefly outlined a concept of the dualism of the porphyrins, stating that in certain pathological conditions increased or decreased Type III porphyrin construction associated with hematopoietic activity may lead to a proportionally increased or decreased Type I porphyrin construction and excretion. This communication presents data which bear out this concept.

A classical case of hemolytic jaundice with 1,600,000 R.B.C., 4.5 gm. of hemoglobin per 100 cc., 21,000 nucleated R.B.C., 5,800 W.B.C., 10% reticulocytes, icterus index 30, Van den Berg (indirect) 3.1 mg.%, and markedly increased R.B.C. fragility was studied. The patient was maintained throughout the period of observation on a bland diet containing small amounts of meat. After 3 blood transfusions splenectomy was performed, following which R.B.C. increased to 2,500,000, hemoglobin to 9.0 gm. per 100 cc., the reticulocytes decreased to 2%, and the icterus index decreased to 10. Porphyrin and stercobilin excretion were followed quantitatively before and after operation.

In the 7 days previous to operation the average daily value for urinary and fecal coproporphyrin was 718 micrograms. The stercobilin averaged 407 mg. during the same period. During the first 6 post-operative days the total coproporphyrin I excretion averaged 648 micrograms per diem. In the same period the stercobilin values fell rapidly to 157 mg. per day. The average coproporphyrin excretion for the next 6 days was 371 micrograms and the stercobilin 73 mg., both within normal range.

These data strongly indicate that physiologically increased hematopoietic activity is accompanied by increased coproporphyrin I excretion. The excretion reverts to normal coincident with decreased hematopoietic activity.

A second patient with hemolytic jaundice, followed for 20 days and not operated upon, showed a daily average total coproporphyrin I excretion of 652 micrograms.

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[1] Dobriner, K., Strain, W. H., and Localio, S. A., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 752.

To prove these data further we investigated quantitatively the coproporphyrin I output in therapeutic hemolysis in a case of polycythemia vera. The patient, a known polycythemia vera with characteristic bluish-red discoloration of the skin and mucous membranes, enlarged spleen, and R.B.C., hemoglobin, hematocrit, blood volume, and viscosity increased to polycythemia levels, received a total of 1.4 gm. of phenylhydrazine-HCl over a 7-day period. This resulted in a decrease of the R.B.C. from 7,700,000 to 4,070,000, hemoglobin from 18.0 to 10.5 gm. per 100 cc., hematocrit from 59 to 36, and viscosity from 7 to 3.8. The icterus index increased from 7 to 30 and the reticulocytes from 1.0 to 5.0%. Throughout the study the patient was maintained on a constant diet.

During the 6 days prior to therapy the average daily total coproporphyrin I excretion was 371 micrograms and the stercobilin 143 mg. No significant changes in porphyrin or stercobilin values were observed during the first 3 days of therapy. In the next 3 days there was however a definite rise of the stercobilin from 148 to 254 mg. a day, the porphyrin values remaining unchanged. On the seventh day of treatment destruction of hemoglobin was manifested by falling blood hemoglobin. During this period the coproporphyrin I excretion rose to 739 micrograms and there was a simultaneous rise in the reticulocytes. The previous elevated stercobilin values reached a peak of 836 mg. These values decreased slightly during the remaining 18 days of observation.

The final evidence was derived from study of a patient manifesting decreased hematopoietic activity. Severe anemia was associated with a neurocytoma of the adrenal with massive metastasis to the bones and destruction of bone marrow. The coproporphyrin excretion averaged only 131 micrograms per day during a 7-day observation period.

Three patients exhibited increased coproporphyrin I excretion during periods of increased hematopoietic activity. One patient showed a return to normal excretion following splenectomy and coincidental with return to normal hematopoiesis. A fourth patient showed a decreased coproporphyrin I excretion associated with decreased hematopoiesis. It is concluded that the production and excretion of coproporphyrin I is parallel to the physiological hematopoietic activity.

9387 P

Excretion of Porphyrin by Dogs.

KONRAD DOBRINER. (Introduced by C. P. Rhoads.)

From the Department of Medicine, The University of Rochester School of Medicine and Dentistry, Rochester, New York, and from the Hospital of the Rockefeller Institute for Medical Research, New York.

Certain qualitative and quantitative studies concerning the excretion of porphyrins have been published previously.[1-5] The existence of a constant ratio between the production of coproporphyrin I and of the Type III porphyrin compounds as reflected by hematopoietic activity has been suggested by those studies. A series of experiments on animals has been made which supports further this suggestion.

The methods of separation and quantitative measurement of the excreted porphyrins are those which have been described previously.[1,2,4] For the studies, fistulæ between the gall-bladder and renal pelvis were made in 4 dogs by the method of Harvey.[6] The animals were fed a constant diet and were kept in metabolism cages. All the urine and feces passed was collected, using toluol or chloroform as a preservative. The blood values were normal according to the figures of Scarborough.[7] Experience has shown that determinations of porphyrin excretion made during periods of less than 10 successive days are valueless.

In all the animals coproporphyrin as well as a small amount of protoporphyrin was excreted in both urine and feces. The coproporphyrin was identified in all instances as coproporphyrin I by melting point determinations. From these facts the dualism of the porphyrins as shown by the constant excretion of a different type of porphyrin from that which is required for the construction of the respiratory pigments is established for dogs.

Quantitative determinations of the total urinary and fecal coproporphyrin excretions were made on 2 normal dogs with bile-renal

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[1] Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

[2] Dobriner, K., *J. Biol. Chem.*, in press.

[3] Dobriner, K., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 175.

[4] Dobriner, K., Strain, W. H., and Localio, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 752.

[5] Dobriner, K., Strain, W. H., and Localio, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 755.

[6] Kapsinow, R., Engle, L. P., and Harvey, S. C., *Sur. Gynec. and Obstet.*, 1924, **39**, 62.

[7] Scarborough, R. A., *Yale J. Biol. and Med.*, 1930, **3** and **4**.

fistulæ. Although the daily total excretion varied greatly, the daily averages of 10-day periods fell within the same range. The first animal was observed from May, 1936, to August, 1936, a total of 110 consecutive days, and showed daily averages coproporphyrin I excretion of 445, 332, 304, 316, 330, 291 micrograms during the first 6 consecutive 10-day periods. The high first value is attributable to the fact that during the first 10-day period the animal was fed dog bile containing coproporphyrin.* During the remainder of the experiment the animal received ox bile which did not contain coproporphyrin. Simultaneous bile-pigment studies showed an average daily total pigment excretion of 92, 83, 86, 97, 128, 118 mg. The second animal showed comparable daily average total coproporphyrin I excretion values for 10-day periods 195, 187, 181, 189 micrograms. Since the animals were of different sizes and the total circulating hemoglobin in the first dog was greater than that of the second animal, hemoglobin construction and destruction in the first animal, although normal, was actually greater. Since the coproporphyrin I excretion seems to be proportional to the total construction of hemoglobin under normal conditions, it is not surprising that the first dog excreted a larger amount of coproporphyrin I. This is similar to the previously reported findings in normal adults and children.

These experiments on animals maintaining a constant hemoglobin level, and consequently a constant respiratory-pigment destruction and construction, show constant daily average total coproporphyrin I excretion. These findings further demonstrate the direct proportion between Type I porphyrin excretion and Type III porphyrin construction in normal individuals.

In order to prove further that coproporphyrin I excretion is not related to blood destruction, but is parallel to normal hematopoietic activity, the following experiment was undertaken. At the beginning of the seventh period the first animal was given a total of 1450 mg. of acetylphenylhydrazine over the period of 18 days. The hemoglobin gradually decreased from 120% at the onset of treatment to 55% on the twentieth day, then gradually rose to 112% during the succeeding 35 days during which time treatment was omitted. Shortly after the administration of phenylhydrazine was begun the bile-pigment excretion increased from a daily average of 118 to 263 and 548 mg. during the two 10-day periods of treatment. The bile-pigment excretion then slowly decreased, averaging 307,

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* About 70% of intravenously administered coproporphyrin I is excreted in 24 hours.

136, and 66 mg. daily respectively during the remaining three 10-day periods without treatment.

Coproporphyrin I excretion remained constant during the first 12 days of phenylhydrazine therapy and then increased suddenly on the thirteenth day to twice its normal value. During the first 10 days of therapy the average was 306 micrograms per diem. There was an increase to 655 and 608 micrograms per day during the eighth and ninth 10-day periods respectively and a decrease to 348 and 269 during the tenth and eleventh 10-day periods. The porphyrin excreted during the period of increased blood construction was characterized as coproporphyrin I. A rapid increase in hemoglobin production was observed simultaneously with the increased porphyrin excretion, making it necessary to increase the phenylhydrazine dosage in order to maintain the anemia level.

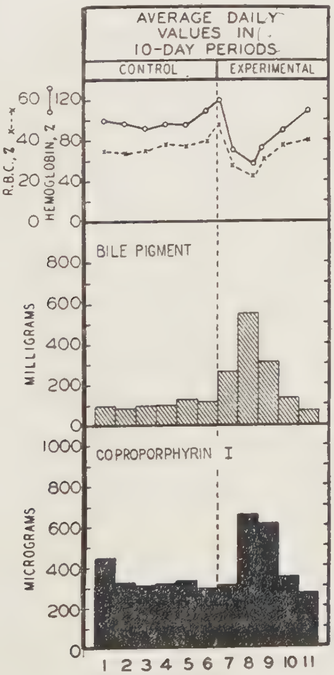

Chart 1.

In chart I the experimental results are summarized and indicate that there is no relation between coproporphyrin-I excretion and blood destruction; on the other hand they demonstrate that there is a very close relationship between coproporphyrin I excretion and hematopoietic activity (Type III porphyrin compound construction).

Conclusions—1. Coproporphyrin I is excreted at a constant rate in normal dogs, proving the existence of the dualism of the porphyrins in that animal. 2. Experimental evidence is given that coproporphyrin I excretion seems to be directly proportional to and a measure of hematopoietic activity. 3. Coproporphyrin I is not a product of or related to blood destruction.

I wish to express my gratitude to Dr. W. B. Hawkins for allowing me to make use of his dogs and to include his blood and bile pigment values in this study.

9388

Inactivation of Bacteriophage by Ethyl Alcohol.

CHARLOTTE A. COLWELL. (Introduced by Arthur I. Kendall.)

From the Department of Research Bacteriology, Northwestern University Medical School, Chicago.

General agreement has prevailed among investigators that phage freed from a major portion of extraneous materials is more susceptible to inactivation by physical and chemical agents than is crude broth phage. One exception to this statement has been noted. Bronfenbrenner[1] reported that phage free from nitrogen as determined by Nessler's reagent or ninhydrin was not weakened in lytic activity when mixed with 10 volumes of alcohol and left for 8 days at 22 to 25°C., although broth phage was inactivated under similar conditions in a very short time. Since Kligler and Olitzki[2] have reported that the presence of protein delays the inactivation of phage by alcohol and Callow[3] found that both purified and broth phages were inactivated by alcohol after 3 days in the refrigerator, corroboration of Bronfenbrenner's findings appears to be worth recording.

Bacteriophage for a strain of *B. coli* was purified from lysogenic cultures by a method recently described.[4] Purified phages used in these experiments contained between 0.3 and 0.5 mg. of nitrogen per 100 cc. as determined by the microkjeldahl method of Koch and McMeekin.[5] Homologous crude broth phage was adjusted to approximately the same titer and pH (6.3). Each kind of phage was mixed with an equal amount of 95% ethyl alcohol and allowed to stand in corked test tubes at room temperature. Titrations of both samples for phage content were made by the serial dilution method in broth every hour for 5 hours and after 16, 18, 24, 48, 72, 96 hours and 5 days.

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[1] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 372.

[2] Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 393.

[3] Callow, B. R., *J. Infect. Dis.*, 1927, **41**, 124.

[4] Colwell, C. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 100.

[5] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

It was found repeatedly that although the titer of broth phage fell to 10^{-1} or 10^{-2} in one hour and completely disappeared in 2 to 4 hours, the purified phage still had a titer of 10^{-6} after 6 hours, 10^{-5} after 24 to 48 hours and 10^{-3} after 72 hours. After 4 days, 2 cc. of the phage-alcohol mixture contained no detectable phage, although the titer of an aliquot of the same phage mixed with sterile distilled water instead of alcohol maintained under the same conditions was still 10^{-7}. Inactivation of the purified phage therefore occurred earlier in this work than in that of Bronfenbrenner previously quoted, owing perhaps to slightly different conditions; but the 2 experiments illustrate the same principle, namely, that purified phage, contrary to general opinion, is more resistant to inactivation by ethyl alcohol than is crude broth phage.

Bronfenbrenner believes the more rapid inactivation of broth phage by alcohol to be due to the precipitation of carrier particles of the phage. When the carrier particles are removed by purification, the phage itself is not so readily inactivated. No alternative explanation of the greater resistance of purified phage to alcohol presents itself at the present time.

TABLE I.
Inactivation of Bacteriophage by Alcohol.

	Titer before alcohol	After 1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Purified phage	10^{-7}	10^{-6} to 10^{-7}	10^{-6} to 10^{-7}	10^{-6}	10^{-6}	10^{-6}	10^{-5}	10^{-5}	10^{-3}	0
Crude broth phage	10^{-7}	10^{-1} to 10^{-2}	0 to 10^{-2}	0 to 10^{-2}	0	0	0	0		

9389 P

Effect of Hydrogen Peroxide on Methemoglobin.

Robert D. Barnard. (Introduced by C. I. Reed.)

From the Laboratory of Therapeutics, University of Illinois, College of Medicine.

Barnard and Gowen[1] reported that the addition of hydrogen peroxide to methemoglobin solutions resulted in the formation of oxyhemoglobin. This change was considered to be in harmony with that deduced from the relative redox potentials of the hydrogen peroxide-oxygen system and the methemoglobin-oxyhemoglobin system.

Keilin and Hartree[2] subsequently showed that the addition of hydrogen peroxide to methemoglobin solutions led to the formation of a substance which they characterize as a methemoglobin peroxide compound.

Because of the discrepancy between the results of Barnard and Gowen and those of Keilin and Hartree the question was reinvestigated. It was found that the observations of the latter investigators could be confirmed when the methemoglobin used was prepared by the oxidation of hemoglobin with ferricyanide. However when methemoglobin is prepared by the oxidation of hemoglobin with quinhydrone the addition to it of hydrogen peroxide results in the formation of oxyhemoglobin. That this latter compound was actually oxyhemoglobin and not a substance with similar absorption bands was demonstrated by treatment with carbon monoxide when CO-hemoglobin resulted.

In a typical experiment, a sample of a 0.5 millimolar oxyhemoglobin solution (molarity in this case refers to iron molarity) is divided into two portions. Each of these portions is placed in the chamber of a double spectrophotometer vessel at the collimator of a Hillger spectrophotometer. The telescope slit of the spectrophotometer is opened to its maximum extent. The photometer is adjusted so that these two solutions cast spectra of corresponding intensities, one above the other—the absorption bands are of course identical since they emanate from identical solutions.

To the contents of the upper chamber, a small quantity of powdered quinhydrone is added, sufficient to change the upper spectrum to that of methemoglobin (about 3 milligrams suffices for the usual contents of one chamber). There are now two distinct spectra, one of methemoglobin above and one of oxyhemoglobin below.

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[1] Barnard, R. D., and Gowen, G. H., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 521.

[2] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc. B*, 1935, **117**, 1.

When a drop of 3% hydrogen peroxide solution is instilled into the methemoglobin solution in the upper chamber, the upper spectrum immediately reverts to that of oxyhemoglobin, identical in intensity and in coincidence of bands with the spectrum of unaltered oxyhemoglobin below.

The passage of illuminating gas through both chambers causes a change of both spectra to that of carbon monoxide hemoglobin and again these bands are identical in position and intensity.

The experiment proves that the compound formed, in this instance, by the reaction of hydrogen peroxide on methemoglobin is actually oxyhemoglobin and not a compound with superficial similarity of absorption bands.

The opinion is advanced that it was a mixture of oxyhemoglobin with the methemoglobin-peroxide compound that led Haurowitz[3] to the statement that the reaction product of methemoglobin and peroxide resembled in its spectroscopic appearance NO-hemoglobin.

9390 P

The Comb of the Baby Chick as a Test for the Male Sex Hormones.

Robert T. Frank and Emanuel Klempner.*

From the Laboratories of the Mount Sinai Hospital, New York City.

This report describes a method of utilizing the 6-day chick in the bio-assay of the male sex hormone, of androsterone particularly. Much of this study was occupied in eliminating pitfalls which obscured assay. Ruzicka[1] first suggested the chick as a test object, utilizing external application to the crest region as described by Fussgänger.[2] The latter claimed that external application of an extract containing only 1/50 of a capon unit gave a reaction as great as 50 times this amount, given intramuscularly to the capon.

The white leghorn responds best. Chicks younger than 6 days show a high mortality. Ten days' treatment has been found the best.

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[3] Haurowitz, F., *Z. Physiol. Chem.*, 1931, **198**, 9.

* Joseph Brettauer Research Fellow in Gynecology.

[1] Ruzicka, M. L., *Bull. Soc. Chimique de France*, 1935, **5**, t. 2, 1497.

[2] Fussgänger, R., *Mediz. u. chem. Abteil. aus den med. chem. Forschungstätten der I. G. Farbenindustrie Act. Ges.*, 1934, **2**, 1934.

One-half-inch mesh screen must be used as flooring to automatically eliminate droppings. Controls must be segregated to avoid both contact and the possibility of swallowing droppings. Failure to avoid this mistake is evident in our earlier controls which showed comb reactions. (See Graph S 1, S 2, S 4.) Only "chick starting mash" and plenty of water are required. Crowding must be avoided. Sick and underweight chicks rarely respond well or uniformly. Ten chicks were found to be an adequate number for each group. Sex of chick plays a minimum rôle, although males tend to give a slightly higher response in comb weight.

Suggested technic. From the 6th day on, groups of 10 chicks were treated once daily with given amounts of crystalline androsterone dissolved in 0.1 cc. of sesame oil (the same sample of sesame oil has been used throughout all experiments). The oil was applied by means of a blunt hypodermic needle attached to a tuberculin syringe, 1/20 cc. along the base of each side of the comb. The amount of oil used is so small that it does not spread or soil the chick. The daily treatments were continued for 10 days. The chicks were then killed with chloroform on the following day, the 17th day after birth.

Immediately after death the chick is weighed. The comb is now removed by making bilateral cuts close to and along the base of the comb down to the skull with a sharp knife. The comb is then freed by undercutting directly on the skull. It is weighed to mg. *at once* to avoid loss of weight by drying. Body weight, comb weight, and sex are recorded. The average comb weight of each group of 10 chicks is recorded graphically.

Sesame oil and Progynon B gave no reaction. Testosterone proprionate and dihydro-androsterone gave some reactions but no attempt at careful titration was essayed in this study. Male urine extracted by the method of Gallagher, Koch and Dorfman[3] gave definite response.

The graphs show high comb weight of the controls in Series 1, 2, and 4, in which no attempt to segregate the controls was made. The opposite applies to Series 3, 5 and 6 in which the controls were carefully isolated.

We are not yet prepared to offer a characteristic curve of response. According to Butenandt[4] 150-200 γ of crystalline androsterone equals one capon unit. The following examples demonstrate

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[3] Gallagher, T. F., Koch, F. C., Dorfman, R. I., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 440.

[4] Quoted from F. C. Koch, *J. Urol.*, 1936, **35**, 382: "Not until 1933 did he (Butenandt and his associates) obtain enough material to establish its formula correctly as $C_{19}H_{30}O_2$ and its potency as 150-200γ per capon unit."

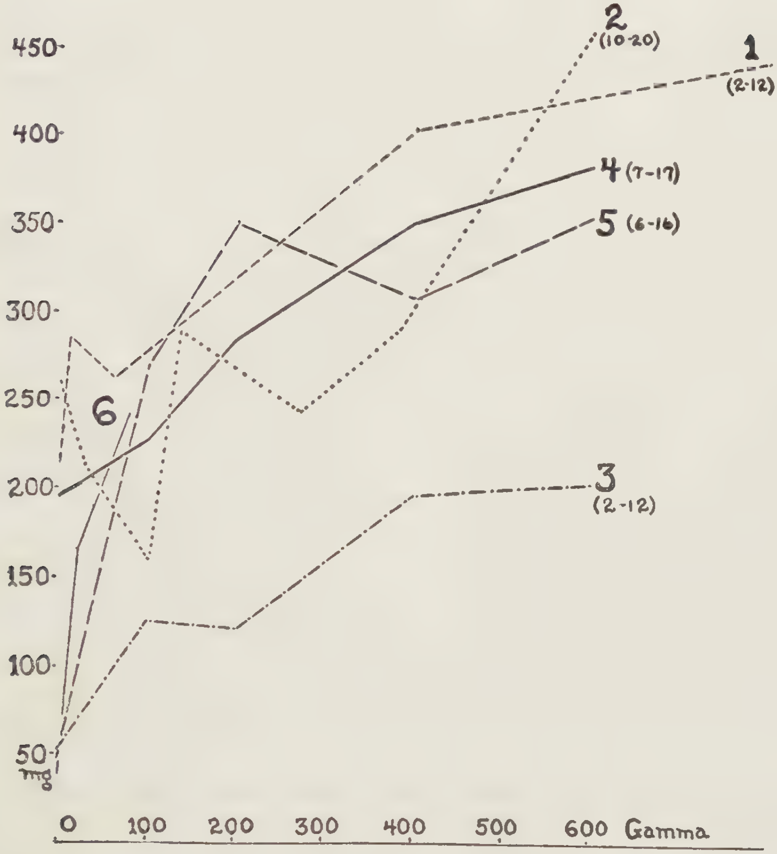

Fig. 1.

the results obtained with this technic: 100 γ androsterone in 10 chicks gave an average comb weight (c.wt.) of 277.1 mg. (heaviest 377, lightest 188); 200 γ androsterone in 10 chicks, average c.wt. 329.7 (heaviest 520, lightest 140). Twenty-seven untreated isolated controls, average c.wt. 49.1 mg. (heaviest 83, lightest 23).

The delicacy of the reaction is evidenced particularly when small dosage is employed (see flattening of ascent above 200 γ of androsterone). Twenty-two untreated controls, average c.wt. 39.3 mg. (heaviest 110 mg., lightest 12 mg.). Ten chicks, total dose 20 γ androsterone (0.1 c.u.) a.c.wt. 164.4 (heaviest 230, lightest 97). Ten chicks, total dose 40 γ androsterone (0.2 c.u.) a.c.wt. 179.5 (heaviest 260, lightest 110). Ten chicks, total dose 80 γ androsterone (0.4 c.u.) a.c.wt. 237.7 (heaviest 380, lightest 170).

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the male sex hormones used in this investigation.

9391 P

Effect of Pituitary Hebin upon Reproductive System of the Chick Embryo.*

L. V. Domm and E. A. Dennis.

From the Whitman Laboratory of Experimental Zoology, the University of Chicago, and the Department of Biology, American University.

In previous experiments we found that, as a result of the daily injection of pituitary hebin in chicks over relatively short periods, testes and ovaries revealed considerable hypertrophy and as a result of this hypertrophy sexual ducts and head furnishings developed precociously.[1] However, in none of the treated female chicks, normal or ovariotomized,[2] did we observe a comparable response on the part of the rudimentary right gonad. In fact this organ revealed very little, if any, growth macroscopically even after relatively prolonged treatment, a rather significant fact in view of the pronounced response by the left ovary. We were, therefore, interested, among other things, in determining whether it was entirely nonresponsive to the gonad-stimulating principle of the hypophysis or whether it could be induced to respond significantly by treatment during the earlier embryonic period either before, or shortly after, morphological differentiation of gonads occurred.

Incubating eggs received single and repeated injections of pituitary hebin, the age of the egg at the time of injection determining whether the hormone was injected into the albumen or into the allantoic cavity. The volume of hormone injected varied from 0.05 to 0.3 cc. and the dosage from 5 to 30 rat units. Injections, which varied from one to 6 in number, were made at varying ages, daily or on alternate days, the earliest beginning at 48 hours and the majority terminating on the 9th day. In a few experiments injections were begun as late as the 12th and 14th day and terminated on the 16th day. Development was allowed to continue until the 18th day when surviving embryos were taken from the shell and examined, and material of interest was fixed for subsequent study. The eggs used were of 2 varieties; pure-bred, single-comb, light brown Leghorn and a hybrid variety resulting from a Rhode Island Red-Barred Plymouth Rock cross. In the latter the sex of the embryo can be determined by a sex-linked plumage character.

A total of 1055 Leghorn eggs were treated from which 218 living

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* This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago.

[1] Domm, L. V., *Transactions on the Dynamics of Dev.*, 1935, **10**, 67.

[2] Domm, L. V., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 356.

embryos were recovered, 101 males and 117 females. Nine hundred and forty-seven hybrid eggs were treated, of which 453 embryos were recovered, 217 males and 236 females. We found that mortality increased with increasing number of injections and with increase in the volume of single injections, but decreased with advancing age of the embryo. The pure-bred Leghorn was found to be less viable than the hybrid variety. The sex ratio of treated embryos recovered showed that females were slightly in excess of males.

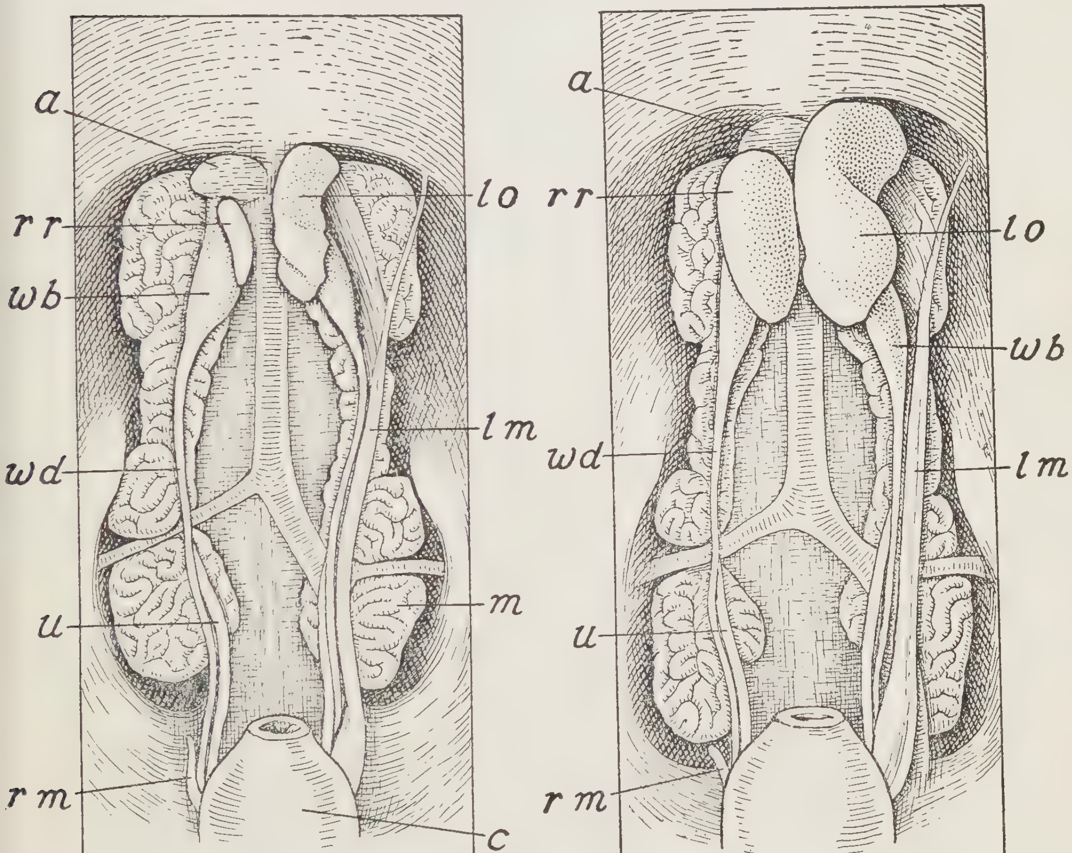

FIG. 1.

Left—Urinogenital system of control female, hybrid embryo, 18th day of incubation. Right—Treated female hybrid embryo of same age which received a total of 25 R.U. of pituitary hebin in 5 equal daily injections. Injections began on 5th day and terminated on 9th. rr—Right rudimentary gonad. lo—Left ovary. wb—Wolffian body. wd—Wolffian duct. lm—Left Müllerian duct. rm—Remnant of right Müllerian duct.

Examination of females revealed hypertrophy of gonads which, though variable, was especially noticeable where higher dosages had been injected. While the response of the ovary was striking and significant, that of the rudimentary right gonad was particularly so since hypertrophy of this organ had not been previously encountered in our post-embryonic pituitary hebin injections. This condition, while also variable, was very pronounced in some of the cases receiving higher dosages. A study of results in the male indicates that while testes have responded by increases in size, they have not responded as frequently nor rarely to the same degree as did the ovary. Gonadal response of both sexes was more pronounced in the hybrid variety than in the pure-bred Leghorn. The age at which injections began did not seem to modify results, for the gonads of both sexes revealed hypertrophy when injections began as late as the 12th or 14th day. Müllerian and Wolffian ducts do not reveal a comparable response; in fact, histological study shows that they have hypertrophied very little, if at all. Likewise, no response could be observed in the head furnishings of either sex.†

A considerable number of the gonads of both sexes have been studied histologically and a brief statement concerning results will be of interest. Treated testes reveal a noticeable hypertrophy of interstitial tissue which is not infrequently vacuolated in character. Tubules are no larger nor more advanced than those of controls. They appear to be less numerous in treated testes due to the greater abundance of interstitial tissue. In some cases small intratubular vacuoles occur in many of the tubules and this combined with a vacuolated interstitial tissue contrasts strikingly with the more dense tissue in control testes.

Histologically, experimental ovaries are characterized by medullary hypertrophy particularly evident in cases receiving higher concentrations. Such cases generally reveal a low, irregular, frequently discontinuous surface layer of cortex with medulla coming to the surface wherever the cortical layer is broken. The medulla consists of numerous medullary tubules, frequently greatly distended, and medullary cords. The right gonads may be divided into 2 types, *viz.*, those which are relatively compact due to preponderance of medullary cords and a more loosely arranged alveolar type exhibiting a preponderance of medullary tubules. The alveolar type may be recognized macroscopically due to its honeycomb appearance. Intermediate types occur. In a number of cases small areas of cortex were encountered on the surface of the right gonad.

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† We recently observed that the head furnishings of the chick embryo may undergo marked hypertrophy when male hormone is injected during the incubation period. Previous studies, by others, had shown that the embryonic sexual ducts of the chick are likewise responsive to introduced sex hormones. It is therefore significant in the present experiments, where gonads have undergone such marked hypertrophy, that sexual ducts and head furnishings reveal no perceptible response.

9392

Permeability of Blood-CNS Barrier in Experimental Poliomyelitis as Determined by the Nitrate Test.*

EDWIN H. LENNETTE AND HAROLD R. REAMES. (Introduced by G. M. Dack.)

From the Department of Hygiene and Bacteriology, The University of Chicago.

The concentration of any substance in the cerebrospinal fluid depends to a large extent upon its concentration in the blood, the passage of such substances between these 2 systems being controlled by a mechanism frequently referred to as the blood-brain barrier. Since variations in barrier permeability patently affect the interchange of constituents of the 2 systems, a number of tests have been devised for measuring alterations in barrier permeability in central nervous system disturbances. One such test is that of Mestrezat and Gaujoux,[1] which depends on the passage of nitrate into the cerebrospinal fluid after its oral administration in the form of sodium nitrate. Mestrezat[2] believes that a high barrier permeability indicates meningeal inflammation. Katzenelbogen[3] states that inflammatory processes within the cerebrospinal canal, as evidenced by pleocytosis and increased protein in the spinal fluid, are frequently associated with high barrier permeability for nitrate, although an increased permeability may occasionally be found in cases with a normal fluid.

To determine if changes in barrier permeability occur in experimental poliomyelitis, we employed a modification of the nitrate test, administering the nitrate intravenously rather than orally. Rhesus monkeys *(Macaca mulatta)* in the preparalytic or paralytic stages of poliomyelitis following intranasal or intracerebral inoculation of the potent MV strain of virus constituted the test group, normal and

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* This work was supported by a grant from the President's Birthday Ball Committee for Infantile Paralysis Research.

[1] Quoted by Loberg, K., *Z. f. d. ges. Neurol. u. Psychiat.*, 1926, **106**, 164.

[2] Mestrezat, W., *Ann. de l'Inst. Pasteur*, 1924, **38**, 719.

[3] Katzenelbogen, S., *The Cerebrospinal Fluid and Its Relation to the Blood*, Baltimore, The Johns Hopkins Press, 1935, 343.

poliomyelitis convalescent monkeys serving as controls. Each animal received intravenously 35 mg. of sodium nitrate per kilo of body weight one hour before cisternal puncture. Every specimen of spinal fluid was tested for globulin by Pandy's method, and the cell content determined. Standard solutions of sodium nitrate containing from 10 mg. to 100 mg. per 1000 cc. were prepared from a 1% stock solution. In performing the test, 2 cc. of Merck's reagent sulfuric acid (NO_3 content 0.0002%) was pipetted into the bottom of clean serological tubes and overlaid with 0.2 cc. of 1% diphenylamine in 75% sulfuric acid. Finally, 0.2 cc. of a standard solution or of centrifuged spinal fluid was added, the 2 top layers carefully mixed and the tubes allowed to stand until a blue ring appeared; the contents were then shaken to obtain a uniform distribution of color and the tubes containing cerebrospinal fluid were compared with the standards.

TABLE I.
Permeability of Blood-CNS Barrier to Nitrate in Experimental Poliomyelitis.

History of Monkey	No. of Monkeys in Group	Concentration of NO_3 in Cerebrospinal Fluid (mg. per 1000 cc.)						
		0-10	10-20	20-30	30-40	40-50	50-60	70-80
Preparalytic	8	1	1	2	3	1		
Paralytic	5	1	1	1		1	1	
Convalescent	6	4	1	1				
Normal	6	2	1	1	2			
Aseptic Meningitis*	3					1	1	1

*Normal monkeys, given 2.0 cc. sterile normal horse serum intraspinally 24 hours before test.

As shown in Table I, the concentration of nitrate in the cerebrospinal fluid varied widely in any one group of animals, ranging from less than 10 to more than 50 mg. per 1000 cc. in the preparalytic and paralytic series, and from less than 10 to more than 30 mg. in the normal and convalescent control animals. The production of an aseptic meningitis by sterile horse serum† introduced intraspinally 24 hours before the test led to the appearance of large amounts of nitrate in the spinal fluid. Although the number of animals tested is small and chance variation in distribution cannot be ruled out, we believe the results indicate that a slight increase in blood-CNS barrier permeability to nitrate may occur in experimental poliomyelitis. The results of spinal fluid examinations for protein and cell content could not be correlated with the nitrate concentration in the fluid.

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† We are indebted to Parke, Davis and Company for a generous supply of normal horse serum.

9393 P

Ultracentrifugal Concentration of a Homogeneous Heavy Component from Tissues Diseased with Equine Encephalomyelitis.

RALPH W. G. WYCKOFF.

From the Rockefeller Institute for Medical Research, Princeton, N. J.

With the demonstration of the existence of a heavy protein in plant tissues diseased with tobacco mosaic virus[1] it became inevitable that similar proteins should be sought in other kinds of virus-infected tissues. Most viruses are more or less unstable towards the chemicals which would naturally be used in efforts to concentrate and purify them. The air-ultracentrifuge[2] has, however, furnished a new tool[3] which has been successful in extracting and purifying several of the less stable virus proteins causing plant diseases[4] and in preparing a similar substance[5] from virus-induced rabbit papillomas (Shope). The same type of ultracentrifugal examination has now been made of tissues diseased with the virus of equine encephalomyelitis.*

As in the previous ultracentrifugal concentrations an angle-centrifuged tissue suspension was ultracentrifuged for one to one and a half hours in a maximum field of ca 50,000 times gravity. All but a trace of the virus activity was thus sedimented. The translucent pellet formed at the bottom of the tube by this ultracentrifugation was resuspended in a suitable solvent and the aggregated colloidal matter thrown down by another low speed centrifugation. Ultracentrifugation of this solution furnished a smaller but cleaner pellet. On occasion the purification of this pellet was carried further by a repetition of the cycle of centrifugation, ultracentrifugation and resuspension. The virus of equine encephalomyelitis is not thermostable; it rapidly becomes non-infectious at room temperature and cannot be relied upon to preserve its activity for long periods of time in the icebox. Therefore all manipulations, including the ultracentrifugations, were carried out without delay and in the cold.

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[1] Stanley, W. M., *Science*, 1935, **81**, 644; *Am. J. Botany*, 1937, **24**, 59.

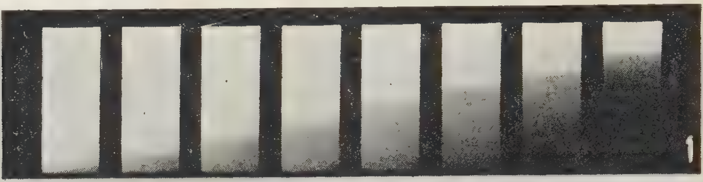
[2] Beams, J. W., and Pickels, E. G., *Rev. Sci. Instr.*, 1935, **6**, 299; Biscoe, J., Pickels, E. G., and Wyckoff, R. W. G., *J. Exp. Med.*, 1936, **64**, 39; Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1936, **64**, 503; 1937, **65**, 565; Wyckoff, R. W. G., and Lagsdin, J. B., *Rev. Sci. Instr.*, 1937, **8**, 74.

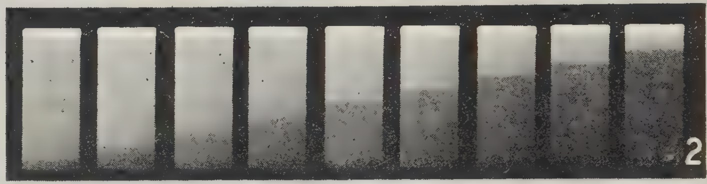
[3] Wyckoff, R. W. G., *Proc. Am. Phil. Soc.*, 1937, **77**, 455.

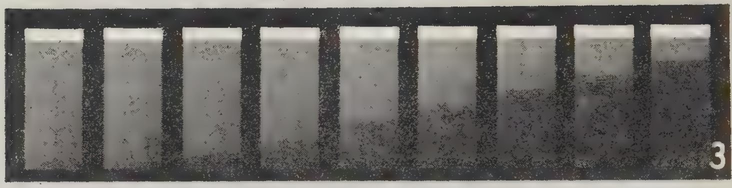
[4] Stanley, W. M., and Wyckoff, R. W. G., *Science*, 1937, **85**, 181.

[5] Beard, J. W., and Wyckoff, R. W. G., *Science*, 1937, **85**, 201.

* The writer is indebted to Dr. Carl Tenbroeck for the infectious material.







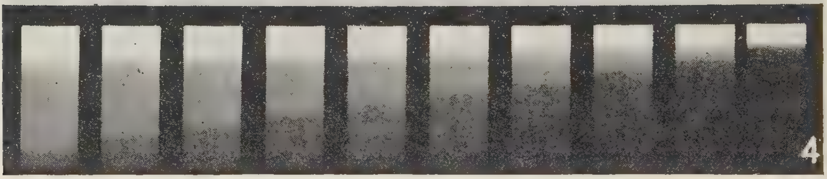


FIG. 1.

A series of exposures showing the sedimentation of the homogeneous heavy component extracted from equine encephalomyelitis-diseased tissue. These pictures were taken every five minutes through a thin layer of the solution lying in a centrifugal field of about 15,000g. The short ultraviolet light used for photography was largely absorbed in the bottom heavy component-containing solution; the liquid above the boundary, freed of heavy particles or molecules, was more transparent. The time rate of descent of the sharp boundary measures the sedimentation constant of the light-absorbing substance.

FIG. 2.

Sedimentation photographs of the solution used for Fig. 1 after it had stood for a few hours at room temperature. The decreased photographic contrast across the sedimenting boundaries is an index of the decreased amount of the heavy component. The greater transparencies of the air bubbles at the very top of the pictures, as compared with the transparencies of the liquids above the boundaries, show the presence of the small ultraviolet-absorbing fragments.

FIG. 3.

Photographs of the solution of the 2 preceding figures after standing somewhat more than a day at room temperature. The heavy component has nearly all disappeared and the heightened contrast between air bubble and liquid above the boundary is indicative of a further increase in the decomposition products.

FIG. 4.

Photographs of another preparation, less pure than that giving Fig. 1. The lower and sharper boundaries are due to the heavy component; the upper diffuse boundaries, which during the series move about one-fourth of the way down the cell, are due to the lighter inhomogeneous material devoid of virus activity.

Both the original suspensions and those resulting from the ultracentrifugations were examined with an analytical ultracentrifuge arranged for photography according to the classical absorption method of Svedberg. A sharp sedimentating boundary of the kind to be expected from a homogeneous heavy protein or other component of a comparable uniformity of particle size was observed when 100 cc. of the especially infectious material obtained by one method of virus cultivation was reduced to 1 cc. through 2 ultracentrifugal steps (Fig. 1). This heavy component was short-lived. After standing for a few hours, less of it appeared in the sedimentation picture (Fig. 2); only a trace was to be seen after a day or so (Fig. 3). Its disappearance corresponds to the breakdown of the heavy substance; the increase in "unsedimentable" material in passing from Figures 1 to 3 demonstrates that its fragments were small and still ultraviolet-absorbing.

If the experiment was not carried out rapidly in the cold and with a definite sequence of suspending fluids the heavy component either was not found or there were present other non-homogeneous, absorbing materials (Fig. 4) in the final concentrated suspension. The nature and origin of such diffusely sedimenting lighter substances are not entirely clear. Similar products have been encountered in the examination of other virus-diseased tissues under conditions which suggest that they arise through disintegration of either the diseased tissue or of the virus itself. Repeated tests have demonstrated that in the present experiments their amount cannot be related to the amount of virus activity; several totally inactive preparations contained nothing else. None of these boundaries, neither the sharp nor the diffusely sedimenting ones, was found when similar healthy tissues were subjected to control ultracentrifugation.

A precise sedimentation constant for the heavy component has not been determined because its solutions have always been somewhat impure. The highest values, $s_{20^\circ} = \text{ca } 245 \times 10^{-13}$ cm. sec^{-1} dynes^{-1}, are probably nearly correct. In less pure preparations where the heavy component sedimented through solutions of lighter proteins, the apparent lowering of s supplied a useful index of the relative amount of this heavy component. From its sedimentation constant, which is of the same order of magnitude as that of the papilloma protein,[5] it is clear that if the particle is spherical and has about the density found for proteins and for the smaller viruses, its molecular weight will be around 25 million; the diameter of such a particle would be about 40 $\mu\mu$.

9394

Sulfanilamide, Antipneumococcus Serum and Vitamin C Therapy in Type II Pneumococcal Pneumonia of Rats.

Frank B. Cooper and Paul Gross. (Introduced by R. R. Mellon.)

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh.

We have reported the successful therapy of experimental Type III and Type I pneumococcal rat pneumonia by the oral administration of sulfanilamide* (*p*-aminobenzenesulfonamide),[1, 2, 3] and also a comparison of sulfanilamide and serum therapy, as well as the combination of both, in Type I pneumococcal rat pneumonia.[3]

The present report is based on experiments with 2 different strains of the Type II pneumococcus and 162 rats. The efficacy of sulfanilamide was tested against these infections and compared with that of Type II antiserum, vitamin C,† and all possible combinations of these substances.

All animals were infected as previously described[2] and treated as indicated under Figs. 1 and 2. Preliminary titrations showed that while strain II-B caused death consistently in rats in a dilution of 1:500 in 1 to 5 days by empyema, bacteremia, and only a minimal pneumonia, dilutions of 1:5 (which were used in this experiment) were necessary to produce a lobar type of pneumonia. Dilutions of 1:1000 of the "Binda" strain regularly produced the desired lesion.

In Experiment 1 treatment was discontinued on the 4th day because most of the controls were dead and the lack of deaths in Groups D, F, G, and H had failed to show any advantage of combination therapy over sulfanilamide alone. Since the deaths that occurred in the next 5 days were evenly distributed throughout these groups, treatment was resumed as indicated.

All rats, including the 15-day survivors, were necropsied and examined as in the previous experiments.[1, 2, 3] The results are shown graphically in Figures 1 and 2.

Sections from the lungs of the fatalities, with the exceptions noted (Fig. 1), showed a lobar type of pneumonia of variable degree and in different stages of development. In the survivors, the infection

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* Kindly supplied by Merck & Co., Inc., Rahway, N. J.

[1] Gross, Paul, and Cooper, Frank B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 225.

[2] Cooper, Frank B., and Gross, Paul, Proc. Soc. Exp. Biol. and Med., in press.

[3] Gross, Paul, and Cooper, Frank B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 535.

† Kindly supplied by Chas. Pfizer and Co., New York.

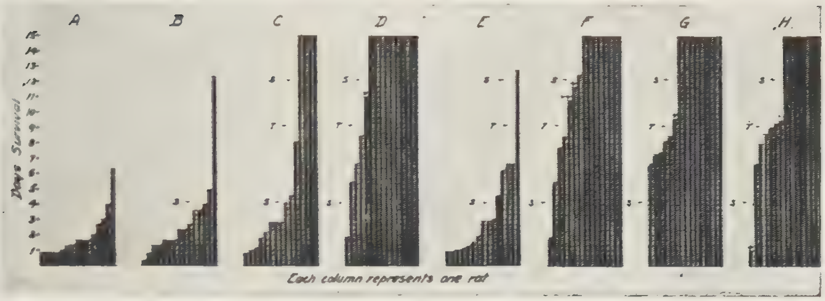

Fig. 1.

Infecting dose: 0.2 cc. of a 1:1000 dilution (in mucin) of an 18-hour broth culture of Strain Binda intrabronchially. A. Untreated control. B. Vitamin C, 10 mg. in water (freshly prepared) twice daily, by mouth. C. 300 units of Type II antiserum intraäbdominally, daily. D. Single dose of 125 mg. sulfanilamide by mouth, daily. E. Combination of B and C. F. Combination of B and D. G. Combination of C and D. H. Combination of B, C and D. All initial treatments given 6 hours after infection. S. Treatments stopped. T. Treatments again started. †No gross evidence of pneumonia. *Enteritis.

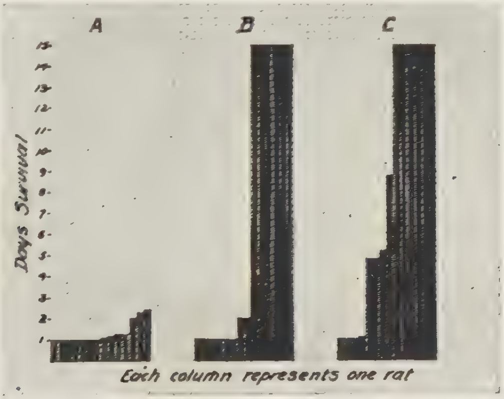

Fig. 2.

Infecting dose: 0.2 cc. of a 1:5 dilution (in mucin) of an 18-hour broth culture of Strain II-B intrabronchially. A. Untreated control. B. 250 units of Type II antiserum, intraäbdominally for 3 consecutive days. C. Single dose of 125 mg. sulfanilamide by mouth, daily, for 9 consecutive days. All initial treatments were given 6 hours after infection.

was represented either by a thickening of the alveolar walls with associated monocytic infiltration, or by a variety of other lesions such as well walled-off abscesses, foci of chronic, organizing pneumonia, and occasionally foci of more recently developed pneumonia.

Experiments with both strains showed that sulfanilamide alone was highly efficacious in reducing the mortality of rats suffering from Type II pneumococcal pneumonia. Compared with the specific antiserum, in the doses employed, the sulfanilamide was at least as effective against heavy doses of Strain II-B, and twice as effective as the serum against a moderate, but lethal, dose of Strain Binda. The average survival time of the rats that ultimately succumbed was 2 to 3 times greater in the groups in which sulfanilamide was given alone or in combination, than in the groups treated only with serum. The combination of any 2 or of all 3 therapeutic agents gave no better results than sulfanilamide alone.

Vitamin C administration alone or in combination with either the serum or the drug, or both, had no beneficial effect in rats; whereas in rabbits, Locke and Mellon[4] observed a marked reinforcement of the drug's action, apparently through stimulation of the phagocyting mechanism. But in mice the sulfanilamide alone appears to overcome hemolytic streptococcal infections largely by its rapid mobilization of the clasmatocytes.[5]

It should be noted that some of the deaths in Groups D, F, G, and H, were probably due to premature termination of the treatments on the 4th day. Many of the rats that died after this time showed pneumonias which appeared to be no older than 48 to 72 hours. Three of the late fatalities in these groups died of intercurrent enteritis and showed a minimal residual pneumonia.

Bacteremia, as judged from stained blood smears, was reduced 50% to 70% in all groups of both experiments where treatment included sulfanilamide. In this respect, serum was effective against Strain II-B, but not significantly so against the Binda strain.

Conclusions. 1. Sulfanilamide was at least as effective as specific antiserum, in the doses employed, in treating Type II pneumococcal rat pneumonia. A probable clinical application to similar human pneumonia is thereby suggested. 2. The combination of sulfanilamide and serum was no more effective than sulfanilamide alone. 3. Contrary to the observations in rabbits (Locke and Mellon[4]), in rats Vitamin C alone, or in any combination tried, is ineffective.

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[4] Locke, A. P., and Mellon, R. R., in press.

[5] Mellon, R. R., and McKinney, R. A., in press.

9395

Growth Rates of Successive Feathers from Single Follicles in the Juvenile Brown Leghorn.*

MARY JUHN.

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

The juvenile plumages of the Brown Leghorn of both sexes pass through a series of changes in pigmentation during successive regenerations. These changes are particularly notable in the feathers which are present at hatching, or emerge shortly after hatching. In the wings and tails, for example, the first feathers to appear are stippled or mottled. The second generation of these feathers shows marked changes towards the adult pattern. In pigmentation, third generation feathers are practically identical with homologous feathers of the adult.

It has been shown previously from this laboratory that there is a direct relation between the concentration of female hormone or thyroxin required for reaction and rate of growth of feathers. (Juhn and Gustavson,[1] Juhn, Faulkner and Gustavson,[2] Lillie and Juhn[3]). There is also a definite range of growth rates characteristic of the feather tracts, and of individual feathers within the tract (Juhn, Faulkner and Gustavson, 1931). These growth relations known to exist in the adult suggest that the juvenile pattern types and changes in these types may be correlated with changes in rates of feather growth during successive juvenile regenerations. In order to test this assumption, we have determined the rates of growth of feathers from a number of tracts in Brown Leghorn chicks. Measurements of length of feathers were begun as soon as possible after hatching, or appearance of feathers, and continued over 2 (in some cases 3) successive regenerations. In all cases which we have studied the rate of growth of the first feather from a given follicle is lowest; second and in many instances third generation feathers grow at increasing rates.

The changes in rates of growth of successive feathers from a single follicle are well illustrated by the main tail feathers. These are present at hatching or appear shortly thereafter. Typical growth

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* This investigation was supported in part by funds from a grant by the Rockefeller Foundation to the University of Chicago in aid of biological research.

[1] Juhn, Mary, and Gustavson, R. G., *J. Exp. Zool.*, 1930, **56**, 31.

[2] Juhn, Mary, Faulkner, G. H., and Gustavson, R. G., *J. Exp. Zool.*, 1931, **58**, 69.

[3] Lillie, Frank R., and Juhn, Mary, *Physiol. Zool.*, 1932, **124**.

curves for first, second, and third generation feathers from the No. 2 follicle (counting from center of body laterally) are shown in Fig. 1. Abscissas represent time in days; ordinates give length of feathers

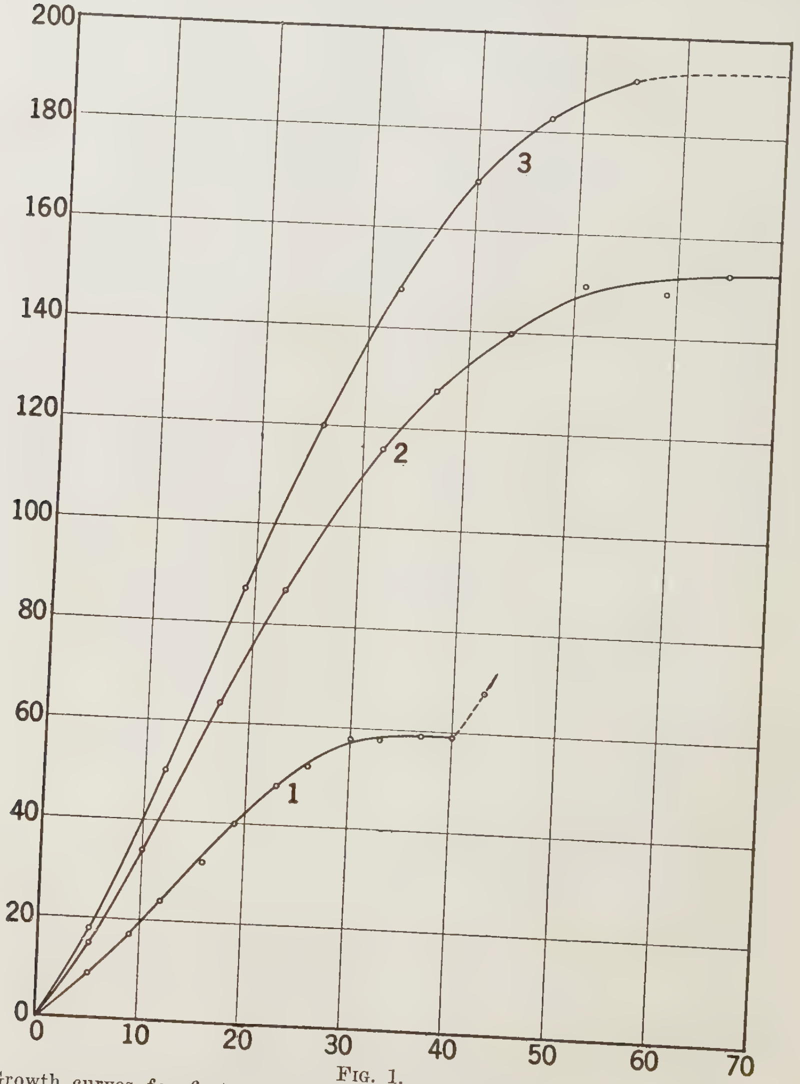

FIG. 1.

Growth curves for first, second, and third regenerates (curves 1, 2, and 3), main tail feather No. 2 (medio-lateral order), right side male Brown Leghorn chick. Abscissas, time in days; ordinates, lengths in mm. The circles represent measured lengths; the common point of origin (0) of the three curves is obtained by transposing individual smoothed curves to a single set of coordinates, taking 0 to be fixed. The increase in length shown by the first feather after completion of its growth (the dotted segment originating on the 40th day) represents growth by the feather of the next generation (curve 2).

in mm. The time of emergence of each feather has been determined by extrapolation of smoothed curves drawn through the indicated points. The numbers on curves (1, 2 and 3) denote first, second, and third generation feathers from the single follicle.

The rates of growth of these feathers expressed in mm. per day are given in Fig. 2, curves 1, 2, and 3 referring to first, second, and third generation feathers. It is clear that there is a rapidly increasing rate of growth at comparable levels of development in successive feathers. A similar order of change has been found in all tail feathers of both sexes, in wing feathers (primaries and secondaries), and in breast feathers.

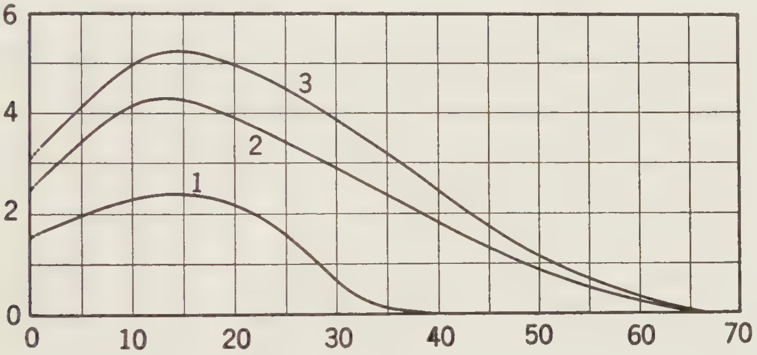

FIG. 2.

Growth rate curves for first, second, and third generations from a single follicle. The rate curves are obtained from the smoothed curves of the preceding figure rather than from the original measurements. Abscissas, time in days from initiation of growth; ordinates, rate of growth in mm. per day.

The change in growth level is much greater between first and second generation feathers (curves 1 and 2 of Figs. 1 and 2) than between second and third generation feathers (curves 2 and 3 of Figs. 1 and 2). This greater shift in growth rate between first and second generation feathers is correlated with a proportionately greater change in pigmentation pattern towards the adult type in these feathers. The first generation feather from the No. 2 follicle of the tail is buff-stippled (or buff-mottled) from apex to base. The second feather from this follicle is typically stippled only at its apex; the remainder of the feather is solid black. The third feather is usually solid black. There is thus a well defined correlation between pigmentation pattern and shifts in rates of growth through the juvenile succession in this particular case.

The length of successive feathers grown from a single follicle increases in approximately the order of shift in growth curves (compare lengths shown by curves 1, 2, and 3 of Fig. 1).

The correlation described here between rate of growth and pigmentation (and feather length) is based strictly upon successive feathers from a single follicle. Feathers composing a given tract, as for example the main tail sequence, do not emerge simultaneously. The last feather of the first generation sequence to appear resembles the second generation feather from the follicle first in order of emergence. The second generation feather of any follicle must accordingly be compared with the first generation feather from that follicle alone. In the breast regions first generation feathers emerge relatively late. These feathers are accordingly much more similar to the adult type than are the first generation tail feathers which bear no resemblance whatever to the black adult feather. In terms of the view previously put forward, the specific pigmentation pattern found in a given feather appears to be a function of its rate of growth. The results described here are taken to support this earlier interpretation of the rôle of growth rates in the determination of pigmentation patterns.

9396

Paralysis in Old Age in Rats on a Diet Deficient in Vitamin E.*

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On January 3, 1935, a group of rats, just weaned, were put on a highly purified fat-free basal diet, 550-B.[1] Supplements consisted of British Drug House vitamin A, viosterol, and yeast from which practically all fat had been removed with alcohol and ether. This yeast proved to be slightly deficient in vitamin B_1 (antineuritic) and was supplemented with 10 gammas daily of crystalline B_1. Fat deficiency symptoms soon developed and were cured by the daily addition of 5 drops of linseed oil. The only factor which was known to be low or absent was vitamin E. The animals (all females) grew moderately well, and at the end of one year had an average weight of 172 gm. During the second year of life 3 of the 8 animals died from unknown causes. The survivors maintained their weight well in spite of the relatively high purity diet. In September, 1936, when approximately 22 months old, all 5 rats became paralyzed with loss of use of their hind limbs. The average weight for the group was 168 gm. Only one rat had lost weight; the rest were at the maximum for their entire life. The most striking fact was the incidence of paralysis at exactly the same age in all rats.

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* The work was aided by grants from National Live Stock and Meat Board and from the Research Fund of the Graduate School of the University.

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

Efforts to effect a cure were made by increasing the yeast, the crystalline B_1, addition of Jensen's B_1 adsorbed on Fuller's earth, and addition of large doses of vitamin E. No method was successful, and in January, 1937, all rats had maintained their weights fairly well in spite of their difficulty in reaching food cups.

The one factor which is known to have been low or absent throughout the experiment was vitamin E. Vitamin E paralysis[2] is readily induced in suckling young and is known to be incurable (except for spontaneous cures). Rats may go through life paralyzed, grow to normal size and produce normal young. Further experimentation may demonstrate that this old age paralysis is also due to vitamin E deficiency. Hitherto it has been thought that a lack of this vitamin had no effect on the adult female rat except to produce sterility.†

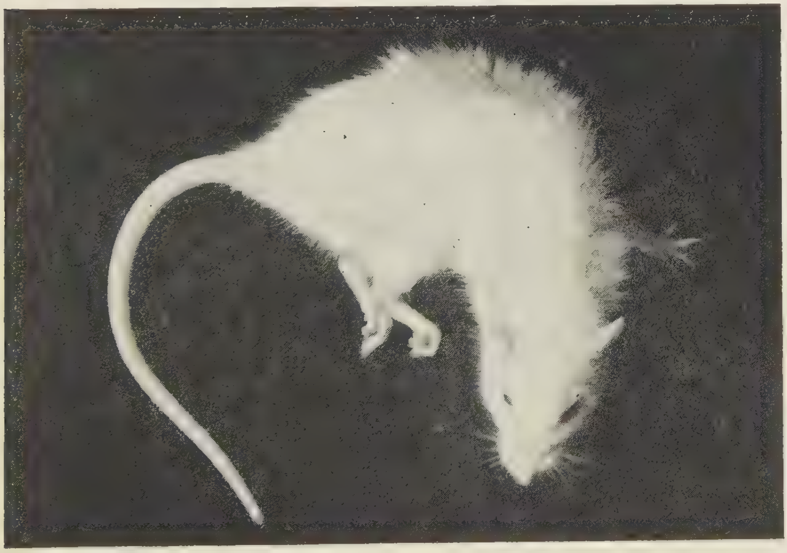

FIG. 1.

Rat No. 34484 photographed at 2 years of age after being paralyzed for 2 months. Weight 176 gm. In fair condition except for paralysis of hind limbs.

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[2] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

† A personal communication from Dr. H. M. Evans of California reveals that a similar paralysis has recently appeared in their colony. They attribute the effect to lack of vitamin E.

The nature of the paralysis is now under study by one of us (R.L.M.).

Gross findings are briefly: (1) In ordinary movements the hind limbs were not used, but were dragged along, in the flaccid condition. However, when the animal made vigorous attempts to move its body, the hind limbs were alternately flexed and extended, but were unable to support the hind part of the body, suggesting a weakness in the adductors of the thigh. (2) Faradic excitation of the skin of the intact animal showed that the sensibility to painful stimuli had not been affected. (3) Faradic excitation of the isolated muscles and larger nerve trunks of the leg and gluteal region gave normal responses. (4) Although normal movement of the hind limbs is affected, the paralysis is neither completely motor nor completely sensory. At autopsy the muscles of the leg and gluteal region were not noticeably atrophic. The sensory ganglia of the brain and spinal cord and the posterior funiculus of the spinal cord had a yellow color. From microscopic examination there are indications of pathological changes which are being studied in detail.

Pathology of the vitamin E paralysis appearing in suckling young has been studied by Lipshutz.[3]

9397 P

Relation between Thyroid and Growth of Testes and Penis When Stimulated by Electric Light.

JACQUES BENOIT. (Introduced by Edgar Allen.)
From the Department of Anatomy, Yale University School of Medicine.

Immature male ducks, when fed with thyroid tissue[1, 2] or injected with thyroxin[2] become sexually stimulated. Furthermore, thyroidectomy delays the normal testicular growth in chickens and ducks[3] and reduces considerably the testicular growth in immature ducks exposed for 15 hours per day during 3 weeks, to the stimulating action of electric light.[4]

In new experiments, 10 Pekin ducks were thyroidectomized and received the same daily illumination during periods of time longer than 3 weeks, in order to establish if the duration of the experiment would influence the response of the testes. Ten ducks were used as control animals.

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[3] Lipshutz, M. D., *Revue Neurologique, T.*, 1936, **65**, 221.

[1] Jaap, R. G., *Poultry Science*, 1933, **12**, No. 5.

[2] Aron, M., and Benoit, J., *C. R. S. Biol.*, 1934, **116**, 218.

[3] Benoit, J., and Aron, M., *ibid.*, p. 221.

[4] Benoit, J., *ibid.*, 1936, **123**, 243.

In order to see if the penis grows as fast in thyroidectomized as in normal animals, some more controls, the testes of which were of approximately the same weight as those of the thyroidectomized animals, were chosen. The weights of their penis were compared in the experimental and control animals.

TABLE I.

Animal No.	Age (mo.)	Exper. (birds treated by electric light)	Duration (days)	Diam. of left testis (mm.)		Wt. of 2 testes at autopsy (gm.)	Thyroid at autopsy (mg.)
				initial	final		
1.							
778	5	controls	33	5.3	34.5	78.63	—
779	5		33	5.3	29.3	58.34	—
780	5	thyroidectomized	32	5.7	9.5	2.33	0
783	5		34	5.3	10.4	2.99	0
2.							
836	4	controls	40	4.7	32.5	73.07	—
837	4		40	5	25.5	30.84	—
830	4	thyroidectomized	41	6	12.7	3.24	0
833	4		41	6	25.7	38.97	0
834	4		41	6	13	3.11	0
844	4		37	5.2	25.4	36.02	0
3.							
823	5	controls	41	10	19	20.22	—
843	4		45	9	32.	61.58	—
807	5	thyroidectomized	47	10	29.2	55.43	1
826	6		41	8.5	27.3	37.3	0
4.							
818	6.5	controls	64	8.5	36.5	90.23	—
829	7		56	6.5	32.	68.27	—
831	6.5	thyroidectomized	61	6.5	29.5	56.77	0
845	6.5		60	9	25	40.25	0

Table I shows that after 32-34 days thyroidectomy still markedly inhibited the growth of the testes, as described in the previous experiments conducted for 3 weeks.[4] This inhibition was diminishing after 37, 41, and 61 days. Testes weighing as much as 38.97 gm. and 56.77 gm. have developed after 41 and 61 days in complete absence of any thyroid tissue.

Prehypophyses from normal ducks treated by light appeared to be rich in gonadotropic factor when implanted into immature female mice.[5] Similar implantation of prehypophyses of thyroidectomized and illuminated ducks showed that these hypophyses were very poor in the gonad stimulating factor as long as the testes were small. The amount of that factor increases when the testes are increasing in size.

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[5] Benoit, J., *ibid.*, 1935, **118**, 672.

The weight of the penis ranged between 0.25 and 0.40 gm. in 4 thyroidectomized ducks whose testes weighed between 2.3 gm. and 3.2 gm. The penis of 4 untreated control animals, with testes of approximately the same weight, (2.3 to 3.7 gm.) weighed from one to 2 gm., that is to say 4 to 5 times more than in the animals under experiment. In experimental ducks having larger testes, that difference exists also but is less marked (ratio = 2.1 to 2.5): Six thyroidectomized ducks with testes from 36 to 57 gm. had penis weighing from 0.9 to 1.7 gm., but the penis of 5 control ducks with testes from 31 to 61.6 gm. weighed from 2.3 to 3.6 gm.

Summary. Thyroidectomy exerts an inhibiting effect on the growth of the testis of the duck stimulated by electric light. This effect is very marked during 3-4 weeks, but diminishes later on. It is not yet possible to say if it disappears completely after a sufficient length of time.

Thyroidectomy exerts also an inhibitory effect on the development of the penis. This inhibition is more marked and more prolonged than that of the testes.

9398 P

Influence of Hypophysis and Thyroid Glands on the Liver of the Duck.

JACQUES BENOIT. (Introduced by Edgar Allen.)
From the Department of Anatomy, Yale University, School of Medicine.

It has been shown previously that the removal of the thyroid glands induces a very marked hypertrophy of the liver in both male and female ducks, mostly through an increase of the lipids, and that the injection of anterior pituitary extract enhances this hypertrophy.[1]

Other experiments were made on 38 ducks (with 33 controls) in order to study the weight of the liver under different experimental conditions:* thyroidectomy, injections of different pituitary extracts, of urinary extract, and of benzogynoestryl[2] with varying

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[1] Benoit, J., *C. R. Ac. Sc. Paris*, 1936, **203**, 468.

* The histological and chemical analysis of the livers obtained in these experiments will be published later.

[2] Benoit, J., *C. R. Ac. Sc. Paris*, 1934, **199**, 1671.

TABLE I.

Experiments	No. of birds	Duration experiment (days)	Wt. of 2 testes at autopsy (gm.)	Liver (gm.)		Ratio $\frac{\text{Liver (gm.)}}{\text{Body (gm.)}}$	
				aver. wt.	extreme wt.	aver. wt.	extreme wt.
I. Pekin Ducks							
♂ Controls (d.l. or D)	17	—	1-10	54.59	36-87	22.84	14 to 36
♂ Controls (e.i.)	14	—	14-144	43.74	33-65	18.23	14 to 24
♂ Thyroidectomized (d.l. or D)	4	32 to 33	0.4-1.3	110	84-140	42.80	28 to 67
♂ Thyroidectomized (e.i.)	18	27 to 61	0.5-94	79.12	45-140	30.96	19 to 50
♂ inj. total extract anter. lobe (sheep) (d.l)		28	1.87	52.84		26.75	
		30	0.52	141.63		42.92	
♂ Thyroidectomized + total extract ant. lobe (sheep) (d.l.)		31	0.78	180.87		72.64	
		31	0.86	63.21		32.92	
♂ inj. Antuitrin-S (d.l.)		20	—	53.31		23.6	
		20	—	31.32		15.3	
♂ inj. Benzogynoestryl (Roussel) (e.i.)		26	0.44	61.4		34.88	
		25	1.28	72.63		33.55	
		27	0.3	73.7		38.8	
		26	13.75	63.47		28.78	
♀ Controls (e.i.)		—	—	81.15		31.82	
		—	—	67.52		31.33	
II. Mallard Ducks							
♂ Controls (d.l.)	879	—	57.35	21.65		13.7	
	880	—	43.46	17.43		17.2	
♂ inj. Antuitrin-S (d.l.)	868	24	57.85	15.95		17.1	
	867	24	o(castrated)	20.71		21.	
♂ inj. Prolactin (d.l.)	873	21	47.7	30.75		28.7	
	874	21	o(castrated)	27.85		27.85	

d.l. = slight daylight (Fall). D = darkness. e.i. = electrical illumination.

degrees of illumination. The size of the testes, which depends upon the intensity of the illumination[2] was shown to be related to the size of the liver.

Table I shows that thyroidectomy is followed by a marked increase in the weight of the liver. On the other hand, this operation delays the testicular growth induced by illumination, as has been shown in another paper.[4] When in the thyroidectomized animals the testes have grown to some extent, the increase of the liver is less marked. Such an inverse relation between testes and liver has been observed in the pigeon by Schooley and Riddle.[3]

Anterior lobe extract induced in 2 ducks a hypertrophy of the liver. Extract of pregnancy urine (Antuitrin S) gave no significant changes, but prolactin did increase the weight of the liver.‡

The injection of pituitary extract seems to have increased the response of the liver in a thyroidectomized duck. A similar result had already been obtained in 2 thyroidectomized animals.[1]

The injection of benzogynoestryl (Roussel) induced an increase of the weight of the liver. Further experiments will show if the female sex hormone secreted by the ovary is responsible for the heavier liver in the female than in the male (*Cf.* table and [1]).

It must be added that neither castration nor hypophysectomy induced an increase in the weight of the liver (unpublished data).

It is probable that the prehypophysis affects the growth of the liver. The hypertrophy of the liver caused by the removal of the thyroids seems due, to a certain extent, to a change in the hypophyseal activity, namely stimulation of the hepatotropic function. Since the development of the testes and penis are lessened by removal of the thyroids[4] and the weights of the liver and testes vary inversely in both normal and thyroidectomized ducks, it is suggested that the gonadotropic and hepatotropic functions of the hypophysis act to balance each other. Finally, the possible interference of some disturbance of the general metabolism after thyroidectomy must not be overlooked.†

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[3] Schooley, J. P., and Riddle, O., *Anat. Rec.*, 1936, **67**, 51.

[4] Benoit, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 782.

‡ The increased weight of the liver after injection of pituitary extracts has already been observed by Carraro (1908), by Cooper and Chamberlain (1925) (posterior lobe extract), Riddle and Flemion (1928), Riddle and Polhemus (1931), Schooley and Riddle (1936) (extracts containing the gonad stimulating factor, or growth hormone, or prolactin), and by Best and Campbell (1936) (anterior lobe extract).

† The writer wishes to acknowledge the supply of extract of pituitary and pregnancy urine from Parke, Davis & Co.; the prolactin from Squibb & Sons, and the benzogynoestryl from Dr. Roussel (Paris).

9399

Hypophyseal Thyrotropic Mechanism Essential for Occurrence of Diabetes Insipidus in its Maximal Form.*

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From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

The contention that d.i. does not occur in the absence of the pars anterior of the hypophysis has been supported by a number of recent investigations.[1] Views differ, however, as to the mechanism of action of the pars anterior in this respect. Barnes and Regan[2] and Biasotti[3] on the basis of their failure to obtain the usual diuretic effect of anterior lobe extract on thyroidectomized dogs suggested that this diuretic effect was by the thyroids through the hypophyseal thyrotropic principle. Mahoney and Sheehan's[4] elimination of d.i. by thyroidectomy strongly suggested that the polydipsia and polyuria of d.i. is dependent upon thyroid action. This concept has been questioned, to a lesser degree, by Gaebler[5] and White and Heinbecker[6] on the basis of their failure to duplicate in all respects Barnes and Regan's and Biasotti's results with anterior lobe extracts and more vigorously by Fisher and Ingram[7] on the basis of their not being able to duplicate, with cats, Mahoney and Sheehan's results on the dog.

Even if one accepts as established that functional thyroid tissue is essential for the appearance of d.i. there still remains the possibility that the thyroid may play its rôle by activation of a hypophyseal diuretic mechanism. In an attempt to elucidate further the rôle played by the pars anterior in d.i. as well as to shed light on several other questions relative to the etiology of d.i. the experiment described and illustrated below was instituted.

The abrupt elimination of a striking, well established polydipsia, by hypophysectomy, has been reported.[1(d)] In this same dog 7½ months after hypophysectomy anterior lobe extract was adminis-

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* Aided by a grant from the Rockefeller Foundation.

[1] (a) von Hann, F., Frankfurt, *Z. f. Path.*, 1918, **21**, 337; (b) Richter, C. P., *Am. J. Physiol.*, 1934, **110**, 439; (c) Ingram, W. R., and Fisher, C., *Anat. Rec.*, 1936, **66**, 271; (d) Keller, A. D., Noble, W., and Hamilton, J. W., Jr., *Am. J. Physiol.*, 1936, **117**, 46.

[2] Barnes, B. O., Regan, J. F., and Bueno, J. G., *Am. J. Physiol.*, 1933, **105**, 559.

[3] Biasotti, A., *Compt. rend Soc. de biol.*, 1934, **115**, 329.

[4] Mahoney, W., and Sheehan, D., *Am. J. Physiol.*, 1933, **112**, 250.

[5] Gaebler, O. H., *Am. J. Physiol.*, 1935, **110**, 584.

[6] White, H. L., and Heinbecker, P., *Am. J. Physiol.*, 1937, **118**.

[7] Fisher, C., and Ingram, W. R., *Arch. Int. Med.*, 1936, **57**, 1067.

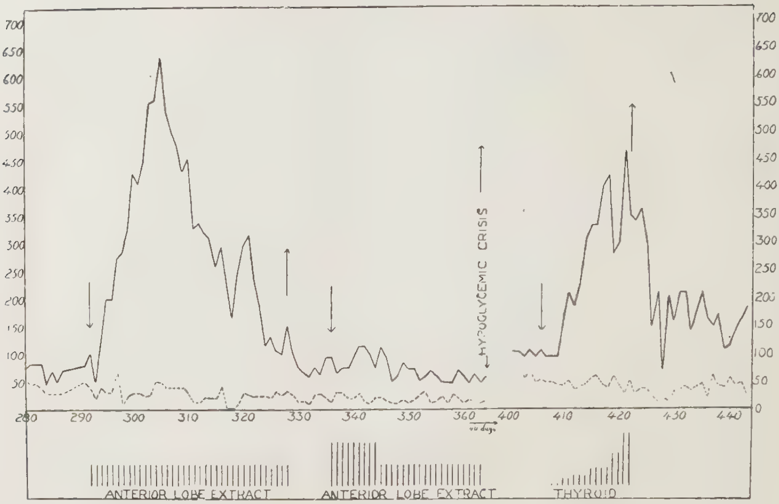

FIG. 1.

Solid line water intake, stippled line food intake (baloration). Ordinate, cc. and gm. per kilo body weight. Dog weighed 4.3 kilo. Abscissa, days after hypothalamic lesion. Hypophysectomy was performed 50 days following the hypothalamic lesion. Anterior lobe extract was administered in 1 cc. and 2 cc. amounts. Thyroid dosage ranged from .1 to 2.4 gm.

tered subcutaneously in order to test the efficacy of this extract in precipitating a polydipsia equivalent to that present before hypophysectomy. The results are graphed in Fig. 1. It is to be noted that the water intake increased after a latent period of 24 to 48 hours but that the increase was progressive over a period of 12 days in spite of a uniform dosage of extract. This is the type of response one would expect if the polydipsia was the result of reactivation of the thyroid. Further, subsequent to the 12th day of extract injections the polydipsia decreased progressively to normal in spite of continued administration of the extract. This result is again exactly the one to be expected if the polydipsia was due to activation of the thyroid by the thyrotropic principle because of the well-established fact that animals soon become thyrotropic-resistant.[8] Note also that the animal was resistant to doubling the dose of extract and that a hypoglycemic crisis resulted in spite of continued injections.

Final verification of direct thyroid action was the subsequent precipitation of a typical polydipsia by thyroid feeding in this same dog.

This experiment, we believe, supports conclusively the contention that a normal functioning anterior lobe of the hypophysis is essential for the appearance of the d.i. in its maximal form which results from derangement of structures in the region of the hypophysis, and that the pars anterior plays its rôle through the thyroid by way of the thyrotropic principle. It also seems justifiable to say that the experiment further establishes d.i. as being due to a deprivation of the antidiuretic principle.[9] Certainly the nature of the experiment and particularly the time that elapsed between operations and extract injections rules out any possibility of the polydipsia being due to irritative phenomena.

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[8] Collip, J. B., and Anderson, E. M., *J. Am. Med. Assn.*, 1935, **104**, 965.

9400

Pathogenic Staphylococci in the Anterior Nares: Their Incidence and Differentiation.*

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From the Laboratory Division of the Hospital for Joint Diseases.

I. INCIDENCE. Because there is no recorded incidence of pathogenic staphylococci in the normal nose a study of their frequency was undertaken in order to establish a standard with which the incidence in infectious conditions might be compared. Such a standard will aid in the interpretation of the reports of Dolman,[1] Danbolt,[2] and Valentine,[3] on the rôle of the nares as a source of infection in recurrent furunculosis.

Cultures were made from the nares of 468 persons without evidence of upper respiratory infection or of staphylococcal infection elsewhere. The staphylococci isolated from these cultures were differentiated into pathogenic and non-pathogenic forms by means of the plasma-coagulation test. This test has been found by various investigators to be a reliable method for such differentiation.[4-8]

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[9] Fisher, C., and Ingram, W. R., *Endocrinology*, 1936, **20**, 762.

* This report constitutes some of the material submitted in partial fulfillment of the requirements for the degree of Master of Science in the Faculties of Teachers College, Columbia University.

[1] Dolman, C. E., *Lancet*, 1935, **1**, 306.

[2] Danbolt, N., *Skrift. Norske Vidensk. Oslo Mat. Natur. Kl.*, 1931, Monograph, 1932; *Biol. Abst.*, 1933, **7**, 1925.

[3] Valentine, F. C. O., *Lancet*, 1936, **1**, 526.

[4] von Daranyi, J., *Cent. f. Bakt.*, 1926, **99**, 74.

[5] Gross, H., *Klin. Wochensh.*, 1933, **12**, 304.

[6] Kemkes, B., *Cent. f. Bakt.*, 1928, **109**, 11.

[7] Chapman, G. H., Berens, C., Peters, A., and Curcio, L., *J. Bact.*, 1934, **28**, 343.

[8] Fisher, A. M., *Bull. Johns Hopkins Hosp.*, 1936, **54**, 393.

Using the results of this cultural study of uninfected persons as a normal standard for comparison, the incidence of pathogenic staphylococci was likewise determined in a group of 51 persons with chronic staphylococcal osteomyelitis, in whom the nares might be considered as a possible focus of infection.

Subjects. The subjects who were cultured, with the exception of a group of 109 adult college students, were patients in the Hospital for Joint Diseases. As previously stated, the group which is considered as uninfected consisted of persons who had no evidence of infection in the nares and who were without evidence of staphylococcal infection elsewhere. The group with chronic osteomyelitis were all proved by culture of the lesion to be infected with staphylococci. If environmental influence is to be considered it should be emphasized that all of the subjects in this series were residents of New York City.

Methods. Swabbings were made from both sides of the nasal cavity and were streaked on plates of beef-extract agar adjusted to pH 7.2. After 24 hours' incubation smears were made of individual colonies and were stained by Gram's method. Staphylococci thus identified were fished and streaked on dextrose-brain-heart-infusion (Difco) agar slants for maintenance of the cultures. The cultures from the children of this series were subcultures from the routine nasal cultures which are taken with throat cultures on all children admitted to the hospital. These were plated on beef-extract agar and were treated as above.

The plasma-coagulation reaction as described by Gross[5] was used as an index of pathogenicity of the staphylococci. To 0.5 cc. of fresh citrated rabbit's blood was added one loopful of a 24-hour agar culture of each strain tested. This was incubated in a water-bath at 37°C. for 3 hours. The reaction was considered positive if a solid plasma-clot was formed within this time. Testing was done on each strain within 4 or 5 days after isolation. The cultural results are shown in Table I.

Comparison of incidence by ages. On comparing the figures shown in Table I it is noted that in all age-groups there is a comparable percentage harboring staphylococci of some kind in the nares. Nevertheless, there is a 20% greater incidence of *pathogenic* staphylococci in the persons under 20 years of age than in those who are older. The age or ages at which this change occurs was not determined because of insufficient data. However, it was found that there is no chronologic decrease (or increase) in the incidence of pathogenic staphylococci in the ages of one through 12 years, but rather a sharp variation around the mean.

TABLE I.
Incidence of Staphylococci in Nares.

Subjects	Total No. cultured	No. and % with staphylococci	No. and % with pathogenic staphylococci	No. and % with non-pathogenic staphylococci	No. and % with no staphylococci
Uninfected		%	%	%	%
Children (2 mo.-12 yr.)	272	201 (73.9)	159 (58.4)	42 (15.4)	71 (26.1)
"Teen" ages (13-19 yr.)	38	30 (78.9)	23 (60.5)	7 (18.4)	8 (21.0)
Adults (over 19 yr.)					
Hospital	49	37 (75.5)	22 (44.9)	15 (30.6)	12 (24.5)
Student	109	87 (79.8)	40 (36.7)	47 (43.1)	22 (20.2)
Total adult	158	123 (78.4)	62 (39.2)	62 (39.2)	34 (21.6)
Staphylococcal Osteomyelitis					
Children	9	9 (100)	4 (44.4)	5 (55.6)	
"Teen" Ages	13	11 (84.6)	7 (53.8)	4 (30.8)	2 (15.4)
Adults	29	22 (75.8)	13 (44.8)	9 (31.0)	7 (24.1)

Incidence in staphylococcal osteomyelitis. In persons under 20 years of age with staphylococcal osteomyelitis there appears to be a tendency to a lower incidence of pathogenic staphylococci in the nares than in uninfected persons of the same ages; whereas the incidence of pathogenic staphylococci in the nares of adults with chronic osteomyelitis tends to be slightly higher than in uninfected persons of the same ages.

Comparison of incidence in children by sexes. There is no difference in the percentage of young boys and girls whose nasal cultures show pathogenic staphylococci, there being 58% of the boys and 56% of the girls having pathogenic forms in the nares. However, in the boys of the "teen" ages there is an incidence of 68% as against 47% in the girls of the same ages.

Effect of hospitalization. In spite of the differences in environment and vigor which existed between the adult subjects of the student and hospital-ward groups, it may be noted that the incidence of all staphylococci as well as the incidence of pathogenic staphylococci are similar in each group.

II. DIFFERENTIATION. Several tests were used to check the results of the coagulase-test of pathogenicity. Sufficient material was gathered to make a comparative evaluation of these tests as means of differentiation. The supplementary tests included chromogenesis, plate-hemolysis, fermentation of mannite, the crystal-violet-agar reaction, and the production of hemotoxin and dermonecrotoxin. The results of these tests, together with their correlation with the plasma-coagulation test, are given below.

The constancy of the coagulase-test. The testing of 233 strains at intervals from one week to one month after isolation showed the reaction to be constant in 94.4% of the strains. The few variable strains more frequently gave a falsely negative than a falsely positive reaction on the first test.

Chromogenesis. Heretofore the differentiation of staphylococci isolated from the nares has been chiefly between the *albus* and *aureus* forms.[9, 10, 11] No further differentiation into pathogenic and nonpathogenic strains was made. In this study the color-chart and arbitrary color-groupings of Winslow and Winlow[12] were used as aids in classifying the colonial color of 215 strains of staphylococci which were isolated from the nares. Of these strains 156 fell into

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[9] Bloomfield, A. L., *Bull. Johns Hopkins Hosp.*, 1921, **32**, 290.

[10] Mackey, L., *Brit. Med. J.*, 1919, **2**, 159.

[11] Williams, A., Nevin, M., and Gurley, C. R., *J. Immunol.*, 1921, **32**, 297.

[12] Winslow, C.-E. A., and Winslow, A. R., *The Systematic Relationships of the Coccaceæ*, John Wiley and Sons, 1908.

the "orange" group. The coagulase-test showed 81% of these to be pathogenic. Of the 53 strains which were in the "yellow" group, 17% were pathogens. Except for 6 "white" strains, all of which were non-pathogenic by the coagulase-test, the pathogenic and non-pathogenic staphylococci covered an identical color-range. Because of this and because such a relatively large percent of the "orange" strains are non-pathogenic we feel that too much reliance should not be placed upon chromogenesis as a means of differentiation.

Plate-hemolysis. In order to determine the relationship of the aerobic hemolytic activity of staphylococci on human blood-agar plates to pathogenicity as indicated by the coagulase-test, 480 staphylococci consecutively isolated from the nasal passages were plated on blood agar. There were 439 strains which showed hemolysis. Among these strains 144 (32.8%) gave negative coagulase-reactions indicating them to be non-pathogenic. Of the 41 strains showing no hemolysis, 20 were pathogenic and 21 were non-pathogenic by the coagulase-reaction.

From these results it would seem that plate-hemolysis *per se* is of little significance. We feel that this reaction as a supplementary means of differentiation has been overemphasized. This is contrary to the opinion of Chapman and his coworkers,[7] who include plate-hemolysis among the important supplementary methods for differentiation.

Fermentation of mannite. The opinion of Hine,[13] Dudgeon and Simpson,[14] and others that the fermentation of mannite with acid-production is indicative of pathogenic staphylococci, and not of non-pathogenic staphylococci, was confirmed in this study. There was agreement with the results of the coagulase-test in 90.97% of the 487 strains which were tested for mannite-fermentation. While the majority of these strains fermented mannite in 24 hours many of them were incubated from 2 to 7 days before fermentation occurred.

Crystal-violet reaction. The crystal-violet-agar method of differentiation of Chapman and Berens[15] was used for the testing of 142 strains of staphylococci of this series. There was agreement in the results of the crystal-violet reaction and the coagulase test in 88.03% of the strains tested.

Production of hemotoxin and dermonecrotoxin. One hundred and thirteen strains of staphylococci were tested for their ability to produce hemotoxin (*alpha* hemolysin) and dermonecrotoxin. Toxins were prepared by a modification of the technic of Parker, Hopkins, and Gunther.[16] Cultures were grown in semisolid agar in an atmosphere of 30% CO_2. After incubation at 37°C. for 48 hours the cultures were centrifugalized at high speed and the supernatants were tested for hemotoxin and dermonecrotoxin. The hemolysis of a 1% suspension of washed rabbit-erythrocytes after incubation for one hour at 37°C. by the supernatants was taken to indicate the presence of hemotoxin. The production of an area of necrosis in the skin of an albino guinea pig following intradermal injection of 0.2 cc. of the supernatants was taken to indicate the presence of dermonecrotoxin.

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[13] Hine, T. G. M., *Lancet*, 1922, **2**, 1380.

[14] Dudgeon, L. S., and Simpson, J. W. H., *J. Hyg.*, 1928, **27**, 160.

[15] Chapman, G. H., and Berens, C., *J. Bact.*, 1935, **29**, 437.

There was no correlation between the production of hemotoxin and the hemolysis of human blood-agar plates.

There were 12 strains which showed the production of strong hemolysin but which showed no dermonecrotoxin. These were equally divided between coagulase-positive and coagulase-negative strains. The remaining 101 strains showed good correlation between the production of hemotoxin and dermonecrotoxin. These also checked with the results of the coagulase-test. This confirms the work of Burnet[17] who stated, "although filtrates having no skin-necrosing action may be active hemolysins, no skin-active filtrates lacking hemolysin have been reported."

Summary. (1) Forty percent of persons over 19 years of age who have no evidence of infection in the nares, or of staphylococcal infection elsewhere, harbor pathogenic staphylococci in the nares. There is a tendency to a slightly higher incidence in persons of the same ages with staphylococcal osteomyelitis. (2) Sixty percent of persons under 20 years of age who have no evidence of infection in the nares, or of staphylococcal infection elsewhere, harbor pathogenic staphylococci in the nares. There is a tendency to a slightly lower incidence in persons of the same ages with staphylococcal osteomyelitis. (3) While the plasma-coagulation reaction was the preferred method of differentiating between pathogenic and non-pathogenic strains of staphylococci, the fermentation of mannite, the crystal-violet reaction and the production of dermonecrotoxin are also reliable confirmatory methods for such differentiation.†

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[16] Parker, J. T., Hopkins, J. G., and Gunther, A., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 344.

[17] Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 717.

† The author is indebted to Professor Jean Broadhurst of Teachers College, Columbia University, and to Dr. John E. Blair of the Hospital for Joint Diseases for valuable suggestions given during the course of this work.

9401

Production of Potent Diphtherial Toxin on a Medium of Chemically Defined Composition.

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As a result of studies carried out by one of us[1] on the chemical nature of the substances required by the bacillus of diphtheria for growth, and by another of us[2] on the factors underlying toxin-production by this organism, it is now possible for the first time to produce toxin of high titer on a medium of known composition. A qualification of the word "known" is admitted in that certain of the components are obtained from natural sources and may contain traces of impurity, and further, that the extremely important traces of certain inorganic elements are beyond control due to the effect of components from the type of glass containers used. The complete absence of peptone, formerly believed to be the *sine qua non* for toxin-formation, appears to be assured.

The composition and method of preparation of the medium is as follows:

glycine	0.5 g.	*beta* alanine	1 mg.
dl-valine	1.0 "	nicotinic acid	2 "
dl-leucine	0.5 "	K_2HPO_4	2 g.
d-glutamic acid hydrochloride	5.0 "	NaCl	5 "
l-cystine	200 mg.	$MgSO_4 . 7H_2O$	0.3 "
dl-methionine	200 "	$CuSO_4 . 5H_2O$	5 mg.
l-tryptophane	100 "	sugar lactate solution containing calcium	20 cc.
l-tyrosine	100 "	distilled water to	1000 "
pimelic acid	1 "		

The glycine, valine, leucine, glutamic acid, methionine and tyrosine, sodium chloride and potassium phosphate are weighed out and dissolved in about 500 cc. of distilled water. The cystine is dissolved in a minimal amount of 1:1 HCl and added to the main lot. The pimelic acid, *beta* alanine and nicotinic acid are now added and the solution adjusted to pH 7.8; 0.3 cc. of a 33% solution of calcium chloride is added and the medium boiled gently for 10 minutes. The precipitated calcium phosphate which carries down any inhibitory excess of iron is now filtered off. Finally the magnesium sulphate, copper sulphate and tryptophane are added and the medium made up to 1000 cc., dispensed and autoclaved. Before inoculation, 2 cc. of a sterile solution containing 37% Merck's U.S.P. sodium lactate, 7.5% Merck's c.p. glucose, 15% purified maltose and 0.3% calcium chloride are added per 100 cc. of medium.

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* Edward Hickling Bradford Fellow, Harvard Medical School.

[1] Mueller, J. H., *J. Bact.*, 1935, **30**, 513; *J. Bact.*, 1937, in press.

[2] Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1936, **17**, 335.

This medium contains approximately 0.7 mg. of nitrogen per cc. The only iron present is that added as impurity in the sugar-lactate solution which fortuitously contains close to the optimal amount of that element.

Toxin-production of 2 Park 8 strains is summarized in Table I.

TABLE I.
Toxin-Production at 35°C.

Strain	Type of flask	Quantity of Medium cc.	Incubation (days)	Lf	L+	M.L.D.
Park No. 8 (Toronto)	Soft glass Fernbach	600	6	36	0.05	.00075
	Pyrex ” ”	600	6	18	—	—
Park No. 8 (Albany No. 5)	125 cc. Pyrex Erlenmeyer	25	4	18	—	.0015
	Soft glass Fernbach	600	7	24	—	.001
	125 cc. Pyrex Erlenmeyer	25	3	13	—	.0025

From Table I it is seen that the Toronto strains produced 36 Lf and 1300 M.L.D. per cc. on a known medium containing only 0.7 mg. of nitrogen per cc. It is noteworthy that this Lf value per milligram of nitrogen in the medium is at least twice that of the most potent toxin hitherto reported.[3] The Albany No. 5 strain produced a somewhat lower titer than the Toronto but growth was less abundant than with the latter strain. It is possible that even higher titers of toxin will be obtained when studies now in progress on additional substances in the tissue-extract fraction as well as on the inorganic elements of the medium have been completed.

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[3] Taylor, E. M., *Ann. Inst. Pasteur,* 1935, **55**, 474.

9402

Dynamics of Collateral Circulation Following Chronic Occlusion of Coronary Arteries.*

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The exact mechanism by which the myocardium is nourished in the presence of chronic obstruction of the coronary arteries is still controversial.[1, 2] The reason for this appears to be that the studies have been largely anatomical and give only qualitative data regarding collateral blood flow.[3] In the normal heart the controversy has been cleared to a large extent by physiological studies. The majority view is best expressed by Wiggers:[4] "The collateral flow is extremely small and insufficient to support contraction in an area rendered [acutely] ischemic by ligation of a main [coronary artery] branch." However, since anatomical connections normally exist between a given coronary artery and (a) other coronary arteries,[5] (b) extracardiac arteries[6] and (c) ventricular cavities,[7] the work of Wiggers does not rule out the possibility of enlargement of existing collateral channels or the development and enlargement of new connections. Indeed, it is known that following acute experimental occlusion of a coronary artery, the infarcted area is generally much smaller than the area originally supplied by the occluded artery.[8] We have, therefore, attempted to devise ways and means of stimulating the development of such an accessory circulation and to study its *physiological extent.*

It seemed to us that the ultimate solution of the problem of the development, source, and determinants of collateral coronary flow might be reached by studying the peripheral coronary pressure (P.C.P.) and blood flow from coronary arteries that have been occluded for varying lengths of time and in hearts with increasing extent of coronary arterial occlusion.

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* Aided by a grant from the Josiah Macy, Jr. Foundation.

[1] Wearn, J. T., *J. Exp. Med.*, 1928, **47**, 293.

[2] Wiggers, C. J., in Levy, R. L., *Diseases of the Coronary Arteries and Cardiac Pain*, Macmillan Co., New York, 1936.

[3] Mautz, F. R., and Beck, C. S., *J. Thoracic Surg.*, in press.

[4] Wiggers, C. J., *Am. Heart J.*, 1936, **11**, 641.

[5] Spalteholtz, W., *Die Arterien der Herzwand*, Leipzig, 1924.

[6] Moritz, A. R., Hudson, C. L., and Orgain, E. S., *J. Exp. Med.*, 1932, **56**, 927.

[7] Wearn, J. T., Mettier, S. R., Klumpp, T. G., and Zschiesche, L. J., *Am. Heart J.*, 1933, **9**, 143.

[8] Smith, F. M., *Arch. Int. Med.*, 1918, **22**, 8.

In the present study chronic coronary artery occlusion was produced in dogs by 2 methods: The right coronary artery or the left descending ramus was occluded by complete ligation of the vessels about one cm. from the aortic ostium. It was noted at the time of ligation that the portion of the ventricular walls supplied by the artery ligated invariably became cyanotic and bulged with each systole. This operation carries a high mortality and preferably should be done in stages. The circumflex branch of the left coronary artery was occluded in 4 stages at the point of origin by means of a silver screw clamp.[9]

Five successful experiments were carried out on hearts 2 to 12 months after complete occlusion of a major coronary artery. After intratracheal ether anesthesia, a combination of heparin, 15 units per kilo and chlorazol fast pink, 0.080 gm. per kilo, was introduced into the circulation to prevent coagulation. A cannula was introduced into the occluded coronary artery just below the point of occlusion and connected to an optical pressure manometer of improved design.[10] Peripheral coronary blood flow was recorded by connecting the P.C.P. manometer to either an Archimedes bucket for mean flow or to a new type of optical recording meter for phasic flow. Aortic and either right or left ventricular pressures were similarly and simultaneously recorded. On exposure of the hearts with remote coronary artery occlusion, the areas which at the time of ligation were cyanotic and bulging now were of normal red color, except for occasional white scars, and definitely showed systolic shortening. Finally, the hearts after death were injected with a barium sulphate gelatin mass using the technique described by Gross.[11] The anatomical increase in collateral communications was determined by Röntgenograms and by dissection of the heart. The degree of infarction was estimated from gross inspection of the sectioned heart.

The criteria for our belief that a considerable collateral circulation develops following chronic occlusion of coronary arteries are: (1) large increases in P.C.P. over normal values, (2) a peripheral coronary blood flow far in excess of normal, (3) a minimum of infarction together with macroscopic evidence of contraction, and finally (4) enlargement of preëxisting or new anatomical connections between occluded and non-occluded arteries.

A portion of this evidence is summarized in Table I. In 4 of 5

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[9] Mautz, F. R., unpublished.

[10] Gregg, D. E., Eckstein, R. W., and Fineberg, M. H., *Am. J. Physiol.*, 1937, **118**, 399.

[11] Gross, L., *The Blood Supply to the Heart*, Paul Hoeber, New York, 1921.

TABLE I.

Data Demonstrating Increased Collateral Circulation After Chronic Coronary Arterial Obstruction.

		Weight		Blood Press. (mm. Hg.) Syst./Diast.						
						Ventricle				
Exp.	Condition	Dog, kg.	Heart, gm.	Aortic	Periph. Cor.	Right	Left	Cor. flow cc./min.	Amt. infarction	Principal collateral (anatomical)
					Right Coronary					
EE25 R31a	Control*	14.0		140/97	30/24	45/1		†		
GM2 R8	Occlusion 8 mos.	8.7	73	80/50	11/10	15/0		0.12	complete	none
GM3 R12	Occlusion 8 mos.	21.2	195	105/62	71/43	32/3		27.0	moderate	Left circumflex
GM4 R4	Occlusion 12 mos.	17.5	181	128/104	110/90	18/1		70.0	slight	,, ,,
					Left Circumflex					
DD36 R21	Control*			110/88	29/18			†		
GM1	Occlusion 4 mos.	11.5	122		109/48		117/5	not taken	slight	Left descendens Right circumflex
					Left Descendens					
DD41 R24a	Control*			88/52	36/22			†		
GM6 R0	Occlusion 2 mos.	8.9	72	82/54	67/40			14.0	moderate	Left circumflex

*These controls were selected because they have P.C.P. values as high as were found in approximately 100 normal control experiments.[2]

† Control flows in excess of 1.5 cc. per min. have not been observed.[12]

[12] Wiggers, C. J., and Green, H. D., *Am. Heart J.*, 1936, **11**, 527.

experiments† there is definite evidence of the development of a collateral circulation following chronic coronary artery occlusion in that (a) the P.C.P. rises to values which approach the aortic pressure, (b) there is a large increase of peripheral blood flow, (c) a minimum of infarction, and (d) an increase in anatomical collateral channels. The extent of this peripheral blood flow with aortic pressures ranging from 82/54 to 128/104 mm. Hg. after complete occlusion of the coronary artery in question is from 14 to 70 cc. per min. The upper range of this volume flow, experiment GM-4, approaches the normal inflow into a coronary artery.‡ Correlating the anatomical data with the blood flow in GM-4, the largest collateral communications found in this series of experiments were present between the right coronary artery and the left circumflex in the posterior A-V sulcus (Fig. 1B as compared with a normal control, Fig. 1A).

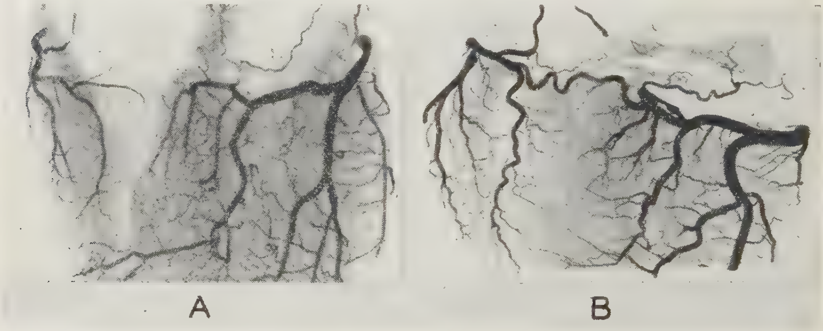

FIG. 1.
B, Röntgenogram showing enlarged collateral channel between right coronary and left circumflex 12 months after right coronary ligation (Exp. GM-4) as compared with normal, A.

In Fig. 2, graphic evidence in the form of reproductions of original P.C.P. curves from the major coronary rami in acutely and chronically occluded arteries is set forth. Inspection of the peripheral right coronary pressure in control A-2 as compared with A-1 shows that following chronic right coronary occlusion, the P.C.P. rises from a probable maximum of 30/24 to 110/90 mm. Hg., a value very close to the prevailing aortic pressure, while its contour

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† We consider experiment GM-2 as our best control. Here there was remote occlusion of the right coronary artery and a collateral circulation failed to develop as evidenced by applying the criteria previously listed.

‡ It should be emphasized that since all volume flows were measured against zero resistance, the actual inflows into the ischemic zone of the intact heart would be somewhat less.

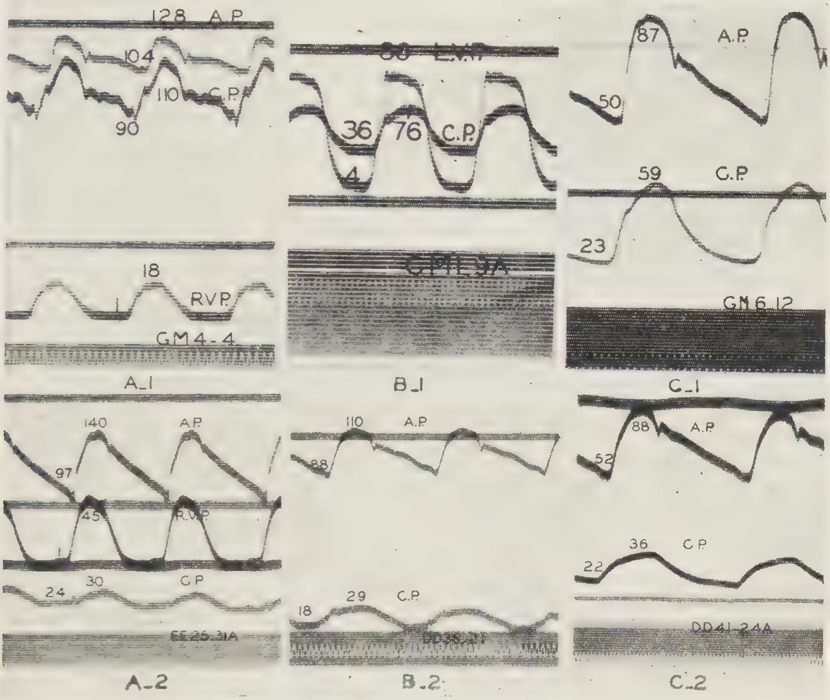

FIG. 2.
A-1, B-1, C-1 are P.C.P. curves after chronic obstruction of right coronary, left circumflex, and left descendens respectively for which A-2, B-2, C-2 are respective controls. AP, aortic pressure; CP, peripheral coronary pressure; RVP, right ventricular pressure; LVP, left ventricular pressure. Time = 0.02 second.

is patterned after the aortic, even showing an incisura. We have always obtained such a rise of the P.C.P. except in experiment GM-2, Table 1, in which collaterals failed to develop and the P.C.P. was 11/10 mm. Hg.

Marked augmentation of the P.C.P. also occurs after chronic occlusion of the left circumflex (B-1 as compared with B-2). The P.C.P. rises from a control value of 29/18 in B-2 to 76/36 mm. Hg. in B-1, or to a value only 10 mm. Hg. below the left ventricular pressure.

Chronic occlusion of the left descendens also shows a definite increase in the P.C.P., C-1 as compared with C-2. Detailed analysis of these pressure curves is reserved for a later publication.

Such experiments, therefore, lead us to the conclusion that following chronic obstruction of a major coronary arterial ramus there develops a massive new collateral coronary circulation. The sources and determinants of such a new myocardial blood supply are now being investigated.

9403

Effect of Diet on Glucose Tolerance of Normal and Hypophysectomized Dogs.

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Kageura[1] reported that dogs utilized glucose at a slower rate than normal following a high fat-low carbohydrate diet. Certain of the animals showed diabetic glucose-tolerance curves. The bearing of the results on problems of therapy in diabetes mellitus seemed of sufficient import to warrant repetition of the experiments.

Accordingly 9 normal dogs were fed for 7 to 14 days on a diet consisting of 100 gm. of fat and 350 gm. raw lean meat daily. Six of the animals were then given 0.85 gm. of glucose *per os* and 3 of them 1.5 gm. of glucose intravenously per kilo of body weight and their glucose tolerance determined by blood analyses made according to the method of Shaffer and Somogyi[2] at the intervals indicated in

TABLE I.
Effect of Diet upon Glucose Tolerance in Normal Dogs.
Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date	Time in min. after administration glucose									Diet	Glucose Tolerance
		0	30	60	90	120	180	240	300	360		
105*	4/24/36	81	111	108	89	82	83	81	87		High CHO	No difference
	5/8	77	97	101	87	80	77	76	83		" Fat	
106*	4/24	85	130	110	82	75	88	87	87		" CHO	" "
	5/8	80	101	118	92	77	74	81	77		" Fat	
107*	2/11	84	116	99	82	92	84	80			" CHO	Improved
	2/17	91	112	155	135	104	93	92			" Fat	
108*	3/30	89	98	97	87	89	85	76			" CHO	No difference
	4/6	75	95	85	79	65	81	78			" Fat	
110*	3/30	88	103	98	88	93	92	94			" CHO	Improved
	4/6	81	113	137	154	142	78	83			" Fat	
A.1*	3/30	93	95	85	88	94	92	91			" CHO	"
	4/6	87	128	144	106	79	90	94			" Fat	
A.5*	2/11	88	93	103	110	99	87	91			" CHO	No difference
	2/17	73	94	106	112	97	77	76			" Fat	
B.38†	2/1/37	76	159	58	83	87	85	83	82	82	" CHO	Improved
	2/16	76	285	185	96	73	65	78	79	80	" Fat	
016†	2/1	80	209	62	71	77	79	81	80	77	" CHO	No difference
	2/16	69	114	72	73	71	80	73	74	73	" Fat	
08†	2/1	76	238	106	82	81	76	73	71	73	" CHO	" "
	2/16	76	225	120	76	72	76	78	78	81	" Fat	

*0.85 gm. of glucose per kg. body weight given *per os*.
†1.5 gm. of glucose per kg. body weight given intravenously.

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[1] Kageura, *J. Biochem.* (Japan), 1922, **1**, 333.

[2] Shaffer, P. A., and Somogyi, M., *J. Biochem.*, 1933, **100**, 695.

the table. The same animals were then given a low fat diet consisting of 100 gm. cane sugar, one liter skimmed milk and 300 gm. whole wheat bread daily for 14 days. Then their glucose tolerance was again determined.

The results are shown in Table I. They demonstrate that American dogs do not exhibit any consistent difference in their glucose tolerance after a high fat-low carbohydrate diet as contrasted with a low fat-high carbohydrate diet. In no instance in this small series was there any decrease in glucose tolerance on a high carbohydrate diet. In some of the animals there was a definite improvement. The suggestion that the difference in response of Japanese and American dogs may be related to a normal difference in their diets naturally presents itself. Japanese dogs are fed on a relatively high carbohydrate diet.

The effect on glucose tolerance of a high fat-low carbohydrate and a low fat-high carbohydrate diet was determined in 8 hypophysectomized animals within 2 months following the operation in the same manner as outlined above for normal dogs. Four of these animals had been previously studied as normal dogs.

TABLE II.
Effect of Diet upon Glucose Tolerance in Hypophysectomized Dogs.
Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date	Time in min. after administration glucose									Diet	Glucose Tolerance
		0	30	60	90	120	180	240	300	360		
111*	2/11/36	88	151	119	73	79	86	83			High CHO	Improved
	2/17	88	143	150	101	85	81	75			’’ Fat	
B.37*	2/11	69	70	72	74	65	69	68			’’ CHO	’’
	2/17	71	82	93	88	71	61	65			’’ Fat	
114*	3/30	77	91	94	77	73	76	78			’’ CHO	’’
	3/16	76	112	116	81	74	67	70			’’ Fat	
A.5*	3/30	89	115	120	71	75	80	85			’’ CHO	’’
	4/6	71	129	156	137	100	69	64			’’ Fat	
B.38†	3/15/37	58	293	144	57	55	67	71	74	76	’’ CHO	’’
	4/5	72	366	256	151	101	66	64	73	83	’’ Fat	
016†	3/15	69	169	60	68	68	66	67	70	71	’’ CHO	’’
	4/5	66	251	79	59	68	77	75	72	77	’’ Fat	
08†	3/15	70	199	49	55	77	73	77	74	81	’’ CHO	’’
	4/5	72	320	189	94	70	65	68	69	70	’’ Fat	

*Glucose *per os.*
†Glucose intravenous.

The results show a striking change from those obtained on normal dogs. (Table II.) All the hypophysectomized animals showed a decreased glucose tolerance on a high fat-low carbohydrate diet.

9404

Effect of Diet on Insulin Response in Normal and Hypophysectomized Dogs.

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In another paper[1] are described the effects of high fat-low carbohydrate and low fat-high carbohydrate diets on the glucose tolerance in normal and hypophysectomized dogs. We report here the effect of similar diets on the insulin response of such animals.

Ten normal dogs were fed from one to 3 weeks on a diet consisting of 100 gm. of fat and 350 gm. raw lean meat daily. The animals were then given 0.5 units of insulin per kilo of body weight intravenously and their response to insulin determined by blood sugar determinations made at the intervals indicated in the table. The same animals were then given a low fat-high carbohydrate diet consisting of 100 gm. cane sugar, one liter of skimmed milk and 300 gm. of whole wheat bread daily for a similar time period. Their insulin response was again determined.

TABLE I.
Effect of Diet on Insulin Response in Normal Dogs.
Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date	Time in min. after administration insulin									Diet	Insulin Response
		0	30	60	90	120	180	240	300	360		
A.1	4/7/36	82	68	49		59	73	91			High CHO	Decreased
	4/1	90	49	48		41	59	92			,, Fat	
105	4/23	83	56	54		53	54	62			,, CHO	Improved
	5/7	66	48	42		60	89	89			,, Fat	
106	4/23	86	51	64		71	88	107			,, CHO	Decreased
	5/7	71	33	44		55	70	73			,, Fat	
107	3/2	88	35	44		42					,, CHO	Improved
	2/21	86	63	47		48						
108	4/1	88	56	56		67	73				,, CHO	Decreased
	4/7	72	61	44		46	64				,, Fat	
110	4/1	98	49	54		79	90				,, CHO	,,
	4/7	77	47	50		55	64				,, Fat	
04	1/29/37	80	45	46	58	73	83	87	87	84	,, CHO	Improved
	2/15	73	58	53	73	81	87	83	83	82	,, Fat	
B.38	1/29	77	64	43	52	63	83	82	84	84	,, CHO	No difference
	2/15	71	52	40	49	67	74	78	75	78	,, Fat	
016	3/8	69	67	31	37	46	63	82	86	84	,, CHO	Improved
	3/30	76	43	40	53	65	76	82	79	76	,, Fat	
08	1/29	78	47	54	66	83	83	89	80	82	,, CHO	No difference
	2/15	73	49	53	55	70	82	86	87	83	,, Fat	

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[1] Weichselbaum, T. E., Somogyi, M., and Heinbecker, P., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 802.

The results are presented in Table I. Their analysis shows no consistent effect of change in diet on insulin response.

Six hypophysectomized dogs within 2 months following the operation were given the above diets for similar time periods and their insulin response again determined. Three of the dogs of this series had been used before in the normal series.

The results are presented in Table II. The animals studied show definitely without exception better insulin response after the low fat-high carbohydrate than after high fat-low carbohydrate diet.

TABLE II.
Effect of Diet on Insulin Response in Hypophysectomized Dogs.
Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date	Time in min. after administration insulin									Diet	Insulin Response
		0	30	60	90	120	180	240	300	360		
B.37	6/1/36	72	38	31		36	58	82	82	88	High CHO	Improved; 0.25U/kg. (Mild convulsions)
	5/7	62	32	30		49	62	68			” Fat	0.5 U/kg.
A.5	4/1	93	41	43		35	33				” CHO	Improved: (Violent convulsions)
	4/7	79	70	46		44	53				” Fat	
B.38	3/8/37	52	45	24	27	34	50	57	56	61	” CHO	Improved
	3/30	69	39	55	65	73	73	73	69	75	” Fat	
016	3/8	70	29	22	33	37	63	69	80	79	” CHO	Improved
	3/30	68	29	27	47	63	76	78	72	72	” Fat	
08	3/8	58	32	30	26	23					” CHO	Improved; (Violent convulsions)
	3/30	71	32	25	28	37	53	72	83	77	” Fat	

A comparison of the effect of similar diets on glucose tolerance in normal and in hypophysectomized dogs shows a definite correlation. In normal animals a change from a high fat-low carbohydrate to a low fat-high carbohydrate diet results either in no change or an occasional instance of improvement of glucose tolerance. The same change of diet produces no clear cut or consistent effect on insulin response. In hypophysectomized animals, on the other hand, there is a markedly increased glucose tolerance after a high carbohydrate-low fat diet and also a consistent and definite improvement in insulin response.

9405

Influence of Protein or Cystine Intake on Cataract-producing Action of Galactose.*†

Helen S. Mitchell and Gladys M. Cook.

From the Division of Home Economics, Massachusetts Agricultural Experiment Station, Amherst.

Ever since it was first demonstrated that galactose causes lens changes in rats[1] experiments have been under way in an effort to explain the metabolic disturbances involved. The basal ration of 15% protein, 70% carbohydrate (galactose and starch), 11% fat (including 2% cod liver oil), 4% salt mixture (Osborne and Mendel), and 0.5 gm. dry yeast 3 times a week, has been modified and supplemented in various ways. The following mineral and vitamin modifications have failed to alter the incidence or speed of cataract development; an excess or omission of salt mixture, drastic shifts in the acid-base balance of the ration, excess or lack of vitamins B and G, crystalline ascorbic acid administered orally or parenterally, massive doses of viosterol. Modifications in the types and amounts of fat or carbohydrate (other than galactose or lactose) have also given negative results. An increase in the amount of protein (15% to 30%) from 3 different sources (casein, lactalbumin, and beef muscle, respectively) also failed to alter the rate of cataract development.

A *reduction* of the *protein* (casein), however, in a 25% galactose ration, from the regular 15% level to 5% level shortened the time of cataract development from 26.3 to 15 days on the average, a difference which is strikingly significant. Furthermore, growth of rats on the low protein ration was limited, as would be expected, and the lower food intake in this group meant less galactose actually consumed, which fact tends to make the aggravating effect of the low protein still more striking. It is worth noting that an *increase* of *galactose* in the basal ration from 25% to 35% is less effective in shortening the time of cataract development than is the lowering of the protein from 15% to 5%.

The loss of cystine and other sulphur-containing compounds from cataractous lenses as reported in the literature[2-4] would suggest that cystine or a related substance may play an important rôle in the

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* Contribution 279 of the Massachusetts Agricultural Experiment Station.

† Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Mitchell, H. S., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 971.

TABLE I.

Cataract Development on a 25% Galactose Ration Modified as to Protein and Cystine Content. (Read across for comparison of litter mates.)

Ration Modification	15% casein Control ration		5% casein		15% casein +1% cystine		15% casein +2% cystine		15% casein +3% cystine	
Litter No.	No. of Rats	Mature Cataract days*	No. of Rats	Mature Cataract days*	No. of Rats	Mature Cataract days*	No. of Rats	Mature Cataract days*	No. of Rats	Mature Cataract days*
67	1	22.0 (21-23)			2	26.8 (21-35)				
68	2	19.2 (18-21)			2	22.0 (18-28)	2	25.2 (21-32)	2	31.5 (25-37)
93	2	24.2 (19-35)	4	13.0 (13-13)						
96	2	47.0 (31-58†)	2	19.2 (15-23)						
97	2	23.2 (16-32)	4	15.5 (13-19)					2	28.0 (26-32)
98	2	21.2 (21-22)	2	16.8 (14-20)					2	25.2 (21-28)
99	2	37.0 (24-49†)	2	17.2 (14-19)			2	40.0 (31-49†)	2	40.0 (28-49†)
101	1	18.5 (18-19)	2	12.5 (12-13)	2	21.2 (19-25)	2	22.7 (21-24)		
102	1	19.0 (18-20)	2	12.2 (11-13)						
103	1	21.0 (20-22)	2	16.0 (13-19)						
104	2	15.2 (15-16)	1	13.5 (13-14)	2	19.0 (19-19)	2	21.8 (19-24)	2	20.2 (19-22)
105	1	20.0 (20-20)			2	24.2 (20-26)	2	22.8 (20-26)	2	27.0 (26-28)
Average days for modified rations groups			21	15.0	10	22.6	10	26.6	12	28.6
Incidence of cataract %				100		100		90		92
			Controls		Controls		Controls		Controls	
Average days for litter mates in each group on the control ration			15	26.3	7	18.4	8	22.6	11	23.0
Incidence of cataract %				87		100		88		91

*Time required for lens changes to become visible to the naked eye (mature cataract). Average and range given for each group.

† Two eyes in each case failed to show mature cataract but did show advanced lens changes at the end of the experimental period. The number of days on the cataract producing ration were used in calculating the time factor for these few animals.

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[2] Fisher, F. P., *Arch. f. Augenh.*, 1934, **108**, 527.

[3] Shoji, Y., *Arch. d'Ophth.*, 1931, **48**, 28.

[4] Weinstein, P., *Arch. f. Augenh.*, 1935, **109**, 221.

metabolism of the lens. Small supplements of cystine used in some preliminary experiments (0, 1-0.25%) gave negative results. Subsequently larger amounts of cystine (1, 2, and 3%) have been employed and the higher levels do appear to have a slight inhibitory effect upon the cataract-producing action of a 25% galactose ration. Bellows' observation[5] of an inhibitory effect when 0.3% cystine was added to a 35% galactose ration is interesting but not convincing because his data are too few and his experiments insufficiently controlled in view of litter differences usually encountered.

The accompanying table is given by litters so that, by reading across, comparisons may be made between litter mates. Each litter was started on experimental rations at 25 days of age. The time differences in cataract development between litter mates on various rations are more striking than average differences. Only figures from litter mate controls have been used in computing averages as given. The phenomenon of cataract-resistant and cataract-susceptible litters, even within a given strain of rats, must be recognized and considered in planning and controlling this type of experiment.

From these data it is evident that a protein deficiency definitely hastens the development of galactose cataract. The relatively slight inhibitory effect of a 20-fold increase in the cystine content of the ration makes it doubtful whether cystine is the crucial factor and leaves the question open as to what other factors may play equal or even more important rôles in this respect.

9406

Serum Protein Changes Occurring in Degenerative Stages of Bright's Disease.

ELOISE JAMESON. (Introduced by T. Addis.)
From Stanford School of Medicine.

Many studies have been made of the changes in serum proteins during the degenerative stages of Bright's disease which are accompanied by edema and a high loss of protein in the urine. In general these studies have been confined to the total serum protein and the albumin-globulin ratio. The results have shown a low total protein and a lowered albumin to globulin ratio.

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[5] Bellows, J., *Arch. Ophth.*, 1936, **16**, 762.

In the work reported here, it has been found that an albumin-globulin ratio does not show the principal changes which have taken place in the serum. Sera widely differing quantitatively and qualitatively as to their component proteins will give similar albumin-globulin ratios. It is necessary, then, to make whole salting out curves to study the differences which occur. These reveal the absence of certain fractions present in normal serum and the presence of another fraction not found in normal serum.

Experiments were carried out on very fresh serum separated from the clot by centrifuging. The serum was precipitated by potassium citrate at 0° and pH 6.8. Under these conditions a minimal amount of change in the serum took place and the results were entirely reproducible. The precipitate was separated from the liquid by filtration. The percents by weight of protein and potassium citrate in the filtrate were determined gravimetrically.

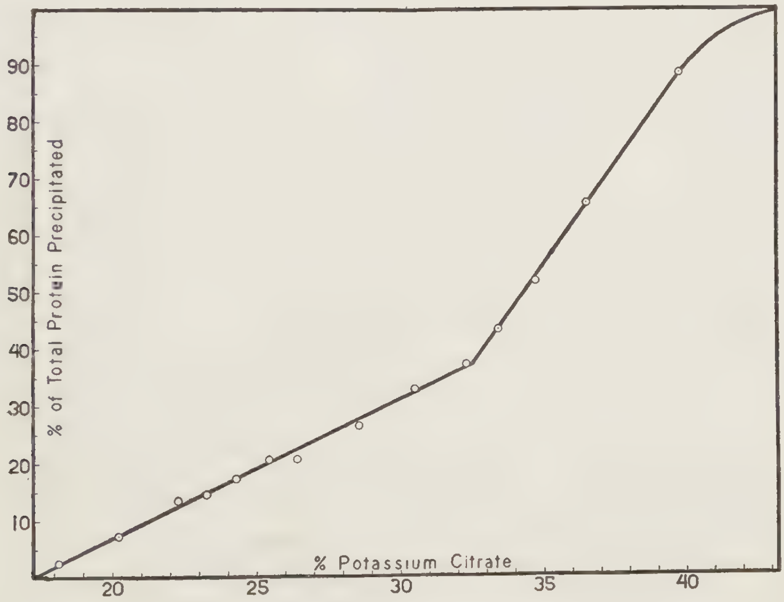

FIG. 1.
Normal human blood serum.

In Fig. 1 is given the normal curve representing the percent of the total protein precipitating, plotted against the percent of potassium citrate. One change in direction occurs, in this case at about 35% citrate. With less of the albumin fraction present in normal serum this point moves to the right. As may be seen, if the curve is cut at 31% citrate a normal 2-1 albumin-globulin ratio is found.

Fig. 2 shows a curve made from the pooled blood serum from 7 cases with glomerular nephritis in the degenerative stage. The total protein was 4.9%. Edema was present in all cases. The most apparent difference from the normal curve is seen from 28 to 31% citrate where a new fraction comes down precipitously. A lessened precipitation in both the extreme globulin and albumin ends is also visible. The albumin-globulin ratio is a little *less* than 1-1.

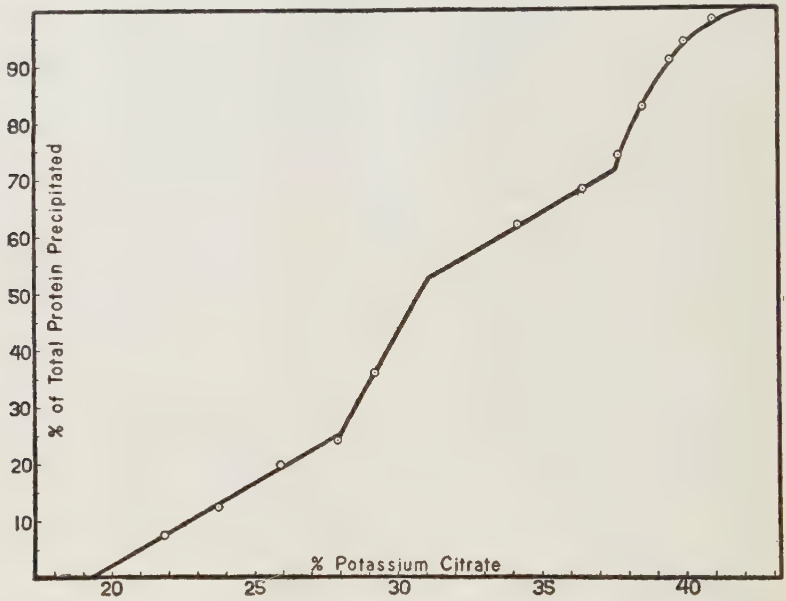

FIG. 2.

Pooled blood serum from 7 cases with glomerular nephritis in the degenerative stage.

The right hand line in Fig. 3 gives the curve from the serum of a case of degenerative Bright's disease during an acute infection (phlebitis). Considerable edema was present. There is a spectacular change both from the normal and from the curve in Fig. 2. The serum proteins are precipitated between 26 and 38% citrate. There is a complete absence of precipitation both in albumin and globulin ends. The albumin-globulin ratio is not far different from that of the serum represented in Fig. 2.

The left hand curve in Fig. 4 gives the results on serum from the same patient during recovery. Precipitation began at 21%. There was a great increase in the globulin end as well as a less but still definite increase in the albumin end. There still remained a large proportion of the fraction coming out precipitously from 27½ to

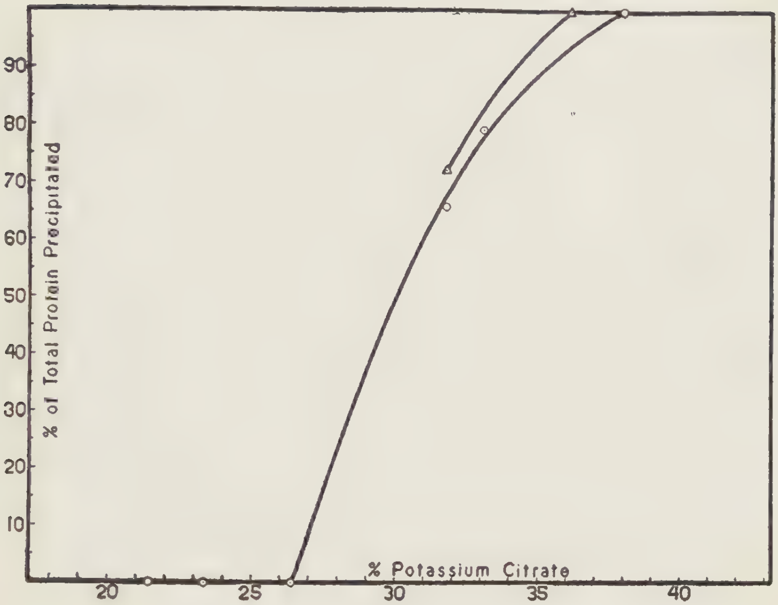

FIG. 3.
Sera from two cases of degenerative Bright's disease during acute infections.

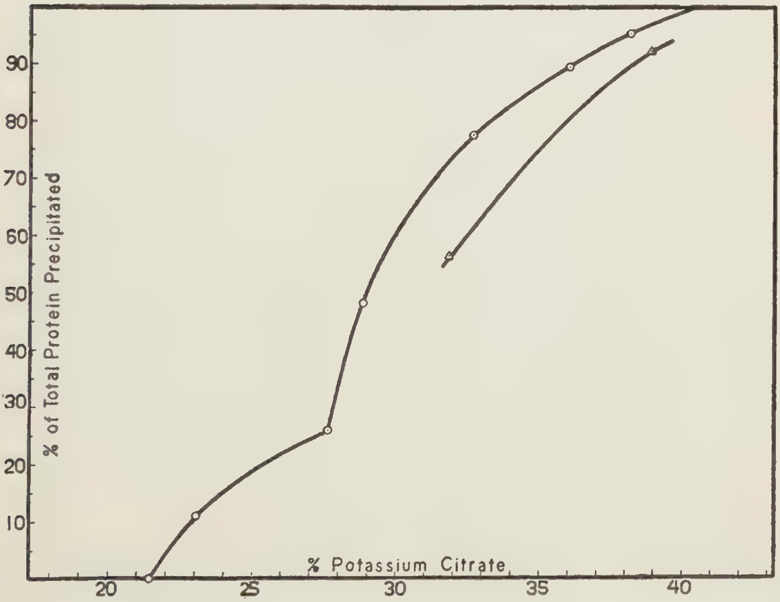

FIG. 4.
Sera from one of the cases of degenerative Bright's disease shown in Fig. 3, before the infection and during recovery.

32½% citrate. Recovery is very apparent although the albumin-globulin ratio is even less than that shown in Fig. 3.

The right hand curve in Fig. 4, made by precipitation of the serum 3 months before the acute infection, indicates that there had been a still greater amount of albumin as well as a greater albumin-globulin ratio at that time.

That the curve in Fig. 3 is not peculiar to one case may be seen from the left hand curve which represents another similar case during an acute infection.

Conclusions. There are 3 obvious changes in a salting out curve of serum of patients in the degenerative states of Bright's disease: (1) Decreased englobulin or absence of it. (2) Decreased albumin. (3) Presence of a fraction not occurring in normal serum. These changes are particularly pronounced where there is an acute infection. They can not be observed by making an albumin-globulin ratio.

9407 P

Importance of the Liver for the Antirachitic Efficacy of Vitamin D.

WALTER HEYMANN.

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The mode of action of vitamin D is at present unknown. A new hypothesis was suggested by Gerstenberger[1] which, on the basis of clinical observations, considered that the action of vitamin D was concerned with some function of the liver thus far unknown. The purpose of the investigation reported here was to approach this hypothesis in animal experiments, in order (1) to study the antirachitic efficacy of vitamin D in rachitic rats in which obstructive biliary cirrhosis had been induced or in which liver damage had been caused by the administration of carbon tetrachloride and (2) to learn whether the resulting decreased antirachitic potency of vitamin D was due to the injury to the liver itself or to other circumstances accompanying the experimental conditions.

Steenbock's rachitogenic Ration 2965 was given to rats 3 weeks of age. To induce cirrhosis of the liver, double ligation and transection of the common bile duct was performed 1 to 1½ weeks after the diet had been started. To induce liver damage, 0.2 to 0.3 cc. of carbon tetrachloride was given by intramuscular injection every second day for 3 consecutive weeks, starting also 1 to 1½ weeks after the rats were placed on the Steenbock ration.

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[1] Gerstenberger, H. J., *Monatschr. f. Kinderh.*, 1933, **56**, 217.

After 3 weeks of the rickets-producing diet (that is, 1½ weeks after operation or after beginning of administration of carbon tetrachloride), antirachitic treatment was begun by intramuscular injection of viosterol in oil, Drisdol, or sodium glycerophosphate solution daily for ten consecutive days.

Under these conditions it was found that bile stasis led regularly in 3 weeks to severe obstructive biliary cirrhosis and that the parenteral administration of carbon tetrachloride resulted in severe fatty and parenchymatous degeneration of the liver rather than in cirrhosis. The results, summarized in Table I, show that 10 to 12 times as much vitamin D (given as viosterol in oil) was needed to cure rickets in rachitic rats having obstructive biliary cirrhosis as was needed in the rachitic controls. This finding confirms similar observations by Greaves and Schmidt.[2] Their interpretation, however, was that the decreased curative efficacy of vitamin D in jaundiced rachitic rats was due (1) to the lack of intestinal absorption of vitamin D caused by absence of bile in the chyme and (2) to a functional impairment of the osteogenic cells caused by the jaundice.

TABLE I.
Curative Doses of Vitamin D and Glycerophosphate Solution in Rachitic Rats with and without Biliary Cirrhosis or Liver Damage.

Groups of rachitic rats used	Vitamin D given by intramuscular injection as				Glycerophosphate solution (10%) given by intramuscular injection	
	Viosterol in oil		Drisdol			
	U.S.P. units	No. of rats	U.S.P. units	No. of rats	cc.	No. of rats
Without biliary cirrhosis or liver damage (controls)	2.5	22	<2.5	14	0.2	11
With biliary cirrhosis	25-30	34	30	44	0.15	11
With liver damage produced by administration of carbon tetrachloride	5-8	63	—	—	—	—

All the curative agents in the author's experiments were given by intramuscular injection; therefore a lack of intestinal absorption does not have to be considered. Furthermore, it was found (Table I) that an even less amount of phosphate solution was needed to produce calcification in the epiphyses of the rats in the jaundiced group than of the controls. It consequently can be concluded that jaundice does not lead to an impairment of the calcifying function of the osteogenic cells. This conclusion is also evident from the results obtained when viosterol was administered to the carbon tetrachloride group in which jaundice, if it was present at all, developed only to a mild degree. That the curative potency of vitamin D is not so markedly diminished in the carbon tetrachloride group as in the rats with cirrhosis probably has to do with the very different kind of pathology produced in the liver by the two methods. In view of the facts that the rats injured by bile stasis or by carbon tetrachloride gained less weight than did the controls and that inhibited growth aids rather than interferes with healing of rickets, it may be said that the reduction in curative effectiveness of the antirachitic agents used would have been even greater if the rats with cirrhosis or those with liver damage had had the same rate of growth as the control animals.

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[2] Greaves, J. D., and Schmidt, C. L. A., *Univ. California Pub. Physiol.*, 1934, **8**, 49.

One further possibility had to be taken into account; namely, that jaundice might have interfered with the absorption of viosterol at the site of injection. Consequently, tests were made with Drisdol, a water-soluble preparation of "pure crystalline vitamin D_2" in propylene glycol that is more easily and more readily absorbed than oil when it is injected into muscular tissue. These results, however, were identical almost unit per unit with those obtained when viosterol in oil was used (Table I).

The only interpretation remaining, therefore, is to conclude that the impaired liver function was responsible for the decreased antirachitic potency of vitamin D, both in the rachitic rats in which cirrhosis of the liver had been induced by ligation of the common bile duct and in those in which degenerative changes of the liver had been caused by administration of carbon tetrachloride. This conclusion substantiates Gerstenberger's[1] hypothesis.

9408 P

Evacuation of the Gall Bladder in Patients with Carcinoma of the Stomach.

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From the Departments of Surgery and Anatomy, University of Minnesota.

This article is the second of a series of 3 studies designed to test the reaction of the human gall bladder to different pathological states of the stomach, *i. e.,* to gastric ulcer, gastric carcinoma and pernicious anemia—diseases in which there are characteristically varying amounts of free hydrochloric acid.

In the first of these[1] it was shown that the gall bladder empties significantly faster in patients with gastric and duodenal ulcers than in normal individuals of comparable age. Although such patients have a high titer of free acid, to which the more rapid emptying of the gall bladder might be attributed, it was found that the curve of emptying in ulcer patients could be duplicated in normal individuals merely by injecting one egg-yolk directly into the duodenum. This would seem to explain the more rapid response of the gall bladder to food in ulcer patients, for it is known that in this disease the hypermotility of the stomach usually results in a greater initial rush of food into the duodenum. Incidentally, these observations remove any apprehension that peptic ulcer may be an antecedent factor in gall bladder disease.

The present report is based upon cholecystographic studies of 22 male patients with carcinoma of the stomach, 13 of them made just prior to gastro-enterostomy or partial resection of the stomach, and 9 of them after one of these operations. In 5 out of the 22 patients (22.7%) the gall bladder failed to visualize, notwithstanding the fact that the dye was administered intravenously. This is a higher negative incidence than occurred in 33 peptic ulcer patients, among whom only 3 (10%) failed to visualize; but this may be explained on the basis of the greater age of the cancer group for, in a previous study,[2] it was found that out of 23 apparently normal men of the 7th and 8th decade, 5 of them (21.6%) failed to become visualized.

Turning now to the rate of emptying of the gall bladder, it was found that in the 10 untreated cancer patients (9 of whom had an average age of 61 years), the gall bladder discharged 74.1% of its contents in the first 40 minutes after a standard meal of egg-yolk.

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[1] Boyden, E. A., and Berman, T. M., *Radiology,* 1937, **28**, 273.

[2] Boyden, E. A., and Grantham, Jr., S. A., *Surg., Gyn. and Obst.,* 1936, **62**, 34.

This is virtually the same as the mean figure previously ascertained for 14 old men[2]—namely 73.3%. It would thus appear that cancer of the stomach (unlike peptic ulcer) does not affect the rate of emptying of the gall bladder.

This is the more noteworthy in that histamine tests failed to elicit the presence of free HCl in 6 of these 10 patients and that the others showed only small amounts; from which it may be inferred that free HCl is not essential to normal emptying of the gall bladder.

Similarly, the presence of some degree of retention of gastric contents in 7 of these 10 patients, following a barium meal, suggests that the length of time it takes the stomach to empty is unimportant, from the standpoint of evacuation of gall bladder bile, so long as there is no delay in the initial discharge of food into the duodenum.

Finally, in 6 cancer patients X-rayed after gastro-enterostomy or partial resection of the stomach, it was found that the rate of emptying of the gall bladder was slightly faster than in normal individuals, the mean discharge being 77.7%. While this difference is not statistically significant, conclusions are withheld pending examination of more cases.

9409 P

Reactions to the Alcohol-Insoluble Fraction of Ragweed Pollen.

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It was shown by Coca and Grove[1] that tryptic digestion of pollen did not destroy its ability to elicit a positive reaction in allergic individuals, although after dialysis no trace of its original nitrogen-content remained. Stull, Cooke and Chobot[2] found that the active principle of ragweed pollen is present in that fraction which is insoluble in 90-95% alcohol. Black[3] by repeated precipitation of ragweed pollen extract in 60% alcohol and redissolving of the precipitate in acid saline, obtained an extract which contained very little nitrogen, and which gave many of the chemical reactions characteristic of polysaccharides. This fraction also gave typical allergic reactions in 43 ragweed-sensitive patients, and no reactions in 50 normals. The highest dilution giving a reaction when injected intradermally was 1:240,000. Unger, Moore, Cromwell and Seeber,[4] in desensitization experiments using both purified protein derivatives and purified polysaccharide fractions, concluded that the specifically active substance in pollen is protein in nature, and that the carbohydrate plays no part in the specificity of their reactions.

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[1] Grove and Coca, PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 48.

[2] Stull, Cooke, and Chobot, *J. Allergy*, 1929-30, **1**, 470.

[3] Black, J. H., *J. Allergy*, 1931, **2**, 161.

To 50 cc. of a 2% aqueous extract of ragweed pollen, 450 cc. of absolute alcohol were added. The resulting precipitate was dissolved in 50 cc. of physiological saline. In control tests, 2% aqueous extracts of pollen were used.

The redissolved precipitate gave typical allergic reactions in individuals sensitive to ragweed pollen. The reaction elicited by the 90% alcohol-insoluble fraction was more intense than that of the 60% alcohol-insoluble fraction, but the type of reaction was essentially the same in each case.

In the allergic individuals reactions were obtained with the 90% alcohol-insoluble fraction in dilutions of 1:10,000,000, administered intradermally. The intensity of this reaction varied in different individuals from a slight reddening to an intense erythema with the formation of a wheal and pseudopods.

It was observed that some apparently normal individuals failed to react to this fraction, while others who gave no reaction to whole ragweed pollen responded to intradermal injections of the alcohol-insoluble fraction.

To confirm this observation, the following series of tests was made: 127 individuals who have never suffered from hay fever were given intradermal injections of whole pollen extract—1:1,000,000—in the right forearm, and of the 90% alcohol-insoluble fraction—1:1,000,000—in the left forearm. These cases were divided into 2 groups—the first composed of those individuals in whose families there were no known cases of allergic disease, and the second, of those cases whose family history was positive for

TABLE I.
Reactions to Alcohol-insoluble Fraction.

	Positive reactions	Negative reactions	Total	% negative reactions
Group I (negative history)	36	38	74	52
Group II (positive ″)	48	5	53	9
Difference in percentage of negative results				43

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[4] Unger, Moore, Cromwell, and Seeber, *J. Allergy*, 1934, **5**, 2.

allergic disease. The results of these tests are shown in Table I. None of these individuals gave a reaction to the whole pollen.

From these data it appears that those individuals who have a family history of allergic disease, but are not themselves sensitive to whole ragweed pollen extract react in a high percentage of cases to the alcohol-insoluble fraction. The ratio of this difference to its standard deviation is 5+, which shows a significant difference exists between the two groups.

Six to 8 hours after the initial injection an area of erythema accompanied by swelling and tenderness, but no wheal formation, was seen to appear. This was first noticed in individuals who failed to react immediately and was later found to occur in those who gave the immediate reaction. This delayed reaction disappeared slowly, in some cases lasting as long as 24 hours.

Attempts at desensitization using the alcohol-insoluble fraction are being carried on.

From these results, it appears that individuals who themselves do not react to whole ragweed pollen extract, but whose family history is positive for allergic disease, give a high percentage (91%) of positive reactions to intradermal injections of the alcohol-insoluble fraction of the same pollen. Those individuals whose family history is negative give a much lower percentage (48%) of positive reactions to the alcohol-insoluble fraction. Possibly some informants, in giving a negative history, were unaware of allergic disease in members of their family; it is probable that many reacting individuals giving a negative family history would be included in the group giving positive histories if complete knowledge were obtainable.

It is also possible that some of the negative reactors might prove to have positive histories, but the statistical analysis of the results obtained in this series makes this seem unlikely.

9410

Renal Oxygen Utilization of Dogs with Experimental Hypertension.*

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From the Departments of Biochemistry and Surgery, Vanderbilt University School of Medicine, Nashville.

It has been shown by Harrison, Blalock and Mason[1-2] and by Friedman and Prinzmetal[3] that a dog kidney rendered ischemic by partial constriction of the renal artery according to the technique of Goldblatt *et al.*[4] contains a greater amount of pressor substance than a normal kidney of either the same or a different dog. Although there is no evidence that this pressor substance (or substances) is responsible for the development of the hypertension exhibited by such dogs, it has been suggested that it is elaborated as a result of some process dependent on ischemia, possibly under conditions of low oxygen tension.

A low tension of oxygen in the renal parenchyma should be reflected in a greater degree of unsaturation of renal venous blood, *i. e.*, by an increase in the renal arterio-venous oxygen difference. In order to see if this were the case, a large number of determinations of this difference was made on normal dogs with a kidney explanted by the method of Rhoads,[5] and similar dogs in which hypertension had been produced by partial constriction of the renal artery. The diet of these dogs consisted of a maintenance allowance of Wayne's dog chow supplemented by meat twice each week. Blood samples were drawn (fasting) by nearly simultaneous puncture of the femoral artery and renal vein without anesthesia.

In Table I the results of 70 observations on 16 normal dogs and 64 similar observations in 4 dogs with partial constriction of the

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* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

† Mr. Evers' assistance in this work was made possible by a grant from the Henry Strong Denison Medical Foundation.

[1] Harrison, T. R., Blalock, A., and Mason, M. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 38.

[2] Harrison, T. R., Blalock, A., Mason, M. F., and Williams, J. W., Jr., *Arch. Int. Med.*, in press.

[3] Prinzmetal, M., and Friedman, B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 122.

[4] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[5] Rhoads, C. P., *Am. J. Physiol.*, 1934, **109**, 324.

TABLE I.
Renal Arterio-venous Oxygen Differences of Normal Dogs and Dogs with Partial Constriction of Renal Artery with and without Hypertension.

	Renal Arterio-venous Oxygen Difference (vol. %)			
	1-2	2-3	3-4	4-5
Normal dogs (70 observations)	12	32	23	3
Dogs with partial constriction of renal artery				
1. Without hypertension (33 observations)	7	15	4	7
2. With hypertension (31 observations)	7	15	5	4
Total	14	30	9	11

renal artery are shown. Thirty-one observations of the latter group were made when the dogs exhibited hypertension, and 33 were made when hypertension was absent in spite of renal artery constriction. It is seen that although a few more rather large arterio-renal venous oxygen differences were encountered in the group with renal artery constriction, the variability in oxygen utilization in this group, with or without attendant hypertension, is not markedly different from that of normal dogs. In both groups the renal A-V(O_2) most frequently encountered was between 2.5 and 3.0 volumes percent.

These results lend no support to the concept that partial constriction of the renal artery is attended by lowered oxygen tension in the kidney, under the conditions specified. Similar observations in circumstances in which the renal excretory work is increased, *e. g.*, a high protein diet, have not as yet been made.

Summary. The variability of renal arterio-venous oxygen difference in fasting dogs with partial constriction of the renal artery with and without hypertension is similar to that of normal dogs.

9411 P

The Active Fraction of Rous Chicken Sarcoma.

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Recent attempts to isolate the active agent of the Rous chicken sarcoma have resulted in conflicting views as to the nature of the causative agent. Jobling and Sproul[1-2] have reported that repeated injections of a lipid fraction obtained from fresh or dried tumor

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[1] Jobling, J. W., and Sproul, E. E., *Science*, 1936, **84**, 229.

[2] Jobling, J. W., and Sproul, E. E., *Science*, 1937, **85**, 270.

tissue, together with nonspecific protein, produce sarcomas which appear in the more responsive birds within 3 to 4 months after the third injection. Fraenkel and Mawson,[3] on the other hand, were unable to obtain tumors by injecting acetone extracts of fresh or dried tumor tissue but were successful with the residue.

In 1936 we attempted to isolate lipid fractions from a very active preparation of Rous chicken tumor No. 1 with pentane and chloroform as solvents. With neither were we successful in producing tumors in young birds. The residue of the pentane extract, however, invariably produced tumors after a single injection in spite of 4 extractions of the powder with 100 volumes of solvent (see Table I). The residue from the chloroform extract was inactive,

TABLE I.
Effects of Injecting Powder, Extracts and Residues of Rous Chicken Sarcoma No. 1 into Barred Plymouth Rock Chicks.

Material	Chick No.	Amt. Inj. mg.	Site	Date of injection	Gain in Wt., gm.	Date Sacrificed	Results
$CHCl_3$	151	100	RL*	6/11/36	340	7/17/36	Neg.
Extd. Pdr.	155	100	RB	"	385	"	"
$CHCl_3$ and	171	75	RB	4/2/37	255	4/24/37	Tumor
Pentane	171	1	LB	"	"	"	"
Extd. Pdr.	171	3	RL	"	"	"	"
	172	25	RB	"	335	4/27	Tumor
	172	15	LB	"	"	"	"
	172	5	RL	"	"	"	"
$CHCl_3$	151	from 400	RB	6/11/36	340	7/17/36	Neg.
Extract	155	" 400	RL	"	385	"	"
$CHCl_3$ and Pentane Extract	175	" 400	LL	4/2/37	370	7/30/36	Neg.
Pentane	152	80	RB	6/3/36	260	6/23/36	Tumor
Extd. Pdr.	156	80	RL	"	105	"	"
	173	75	RB	4/2/37	255	4/22/37	"
	173	1	LB	"	"	"	"
	173	3	RL	"	"	"	"
	174	25	RB	"	155	4/19	"
	174	15	LB	"	"	"	"
	174	5	RL	"	"	"	"
Pentane	152	" 250	RL	6/3/36	260	6/23/36	Neg.
Extract	156	" 200	RB	"	105	"	"
	175	" 300	RB	4/2/37	370	7/30	"
Chicken	176	75	RB	"	235	4/17/37	Tumor
Tumor No. 1 149†	176	5	LL	"	"	"	"

*RB—Right breast. LL—Left leg, etc.
†Controls in 10 additional birds are not included in table.

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[3] Fraenkel, E. M., and Mawson, C. A., *Nature*, 1937, **139**, 282.

possibly due to denaturing the protein. The whole powder, used as a control, was very active; birds had to be sacrificed 12 days after injection. We then believed that the active fraction was not to be found among the lipids; that it was water soluble and very likely protein in nature.

In view of the recent report of Jobling and Sproul,[2] we repeated our experiments with a portion of the powder previously used. It was kept at about 5°C. for 11 months in a sealed but not evacuated tube. Pentane was used for extraction in one series of experiments; a mixture of equal volumes of chloroform and pentane in another. In each case 0.5 gm. powder was extracted 4 times with 100 volumes of solvent, taking measures that would tend to avoid denaturing the protein. The extractions were made with anhydrous solvents for one-half hour each *in vacuo* at 0°-5°C., shaking every 5 minutes. The powder was centrifuged off each time and after the last extraction dried in a vacuum desiccator. The 4 pentane extracts were combined, filtered clear, the pentane distilled off *in vacuo* and the fat taken up in one cc. of sterile olive oil. The 4 chloroform-pentane extracts were similarly treated. In other experiments the tumor powder fat was partly emulsified with an aqueous solution of egg albumen, gelatin or chicken muscle powder.

The extracts from 200 to 400 mg. of powder were injected into muscles of birds. Daily observations showed the injected fat could no longer be palpated after 6 days. Autopsy 3 months later confirmed these negative results. The extracted powders, rubbed in sterile salt solution, were injected into various muscles of birds in doses equivalent to 75, 25, 15, 5, 3 and one mg. With every dose, the residue from both extracts produced definitely palpable masses within a week. The size of the tumors was proportional to the dose administered. Birds injected with the residue of the pentane extract developed larger tumors more rapidly than those in which the chloroform-pentane residue was used. With pentane residue, the tumors were so large and hemorrhagic that these birds had to be sacrificed from 17 to 25 days after injection.

Our observations of last year are thus confirmed and extended. Typical Rous chicken sarcomas have been produced with as little as one mg. of pentane or chloroform-pentane extracted chicken tumor No. 1 powder. Tumors may probably be produced with considerably smaller amounts of these residues.

Additional indication that the activity lies in the protein fraction was obtained by heating the tumor powder, one portion dry and another with 50 volumes of water, for 30 minutes in evacuated flasks in a bath kept at 60°C. Both flasks were evacuated and then filled with nitrogen 4 times and finally evacuated before heating. The dry heated powder when rubbed up with saline and injected into the breast and leg muscles in doses of 40 and 10 mg. respectively in each of 2 chicks produced 4 tumors, whereas the powder subjected to the same conditions of heating in the presence of water, when similarly injected in 2 birds gave negative results. In other words, when the protein was coagulated the activity was lost while when denaturing of the protein was limited by dry heat, activity was retained.

This preliminary work would seem to indicate that the active agent is water soluble and probably resides in the protein fraction.

9412 P

Effects of Androgenic Substances in the Female Rat.*

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From the Department of Anatomy, Wayne University College of Medicine.

Butenandt[1] and others (see Koch[2]) have reported that some of the unsaturated androgens, notably androstene-dione and dehydroandrosterone, will induce estrus in the normal infantile rat. Browman[3] reported the cessation of estrous cycles in adult normal rats injected with androsterone and testosterone. In spayed rats Nelson and Gallagher[4] found that androstane-diol and androstene-dione failed to produce vaginal cornification, but did induce uterine and mammary hypertrophy and prevented castration changes in the hypophysis.

The administration of testosterone† (0.5 and 1.0 mg. daily), androsterone (1.5 mg. daily), dehydroandrosterone (1.0 mg. daily) and androstane-dione (1.0 mg. daily) to groups of 2 to 4 spayed female rats for 30 days failed in every case to produce vaginal cornification (as determined by daily smears and histological sec-

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* This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Butenandt, A., *Die Naturwiss.*, 1936, **24**, 15.

[2] Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.

[3] Browman, L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 205.

[4] Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

† The writers are very grateful to Dr. T. F. Gallagher and Dr. F. C. Koch for their kindness in preparing and furnishing us with the synthetic androgens used in this study.

tion). In all cases castration changes were completely or almost completely prevented. Testosterone and androstane-dione in all instances induced definite mammary proliferation and enlargement of the uterus. Androsterone and dehydroandrosterone failed to induce changes in the mammary glands or uterus.

The above androgens, and in addition, androstene-dione, cis-androstene-diol and trans-androstene-diol have been administered to groups of 2 to 5 adult normal female rats for 16 to 30 days. Daily vaginal smears were made, in each instance, for at least 2 weeks prior to the initiation of and throughout the period of treatment. Normal 4 to 5 day cycles were present in all animals prior to treatment.

Testosterone at the one mg. daily level completely suppressed cycles in one and partially suppressed them in 2 rats. At the 2.0 mg. daily level cycles were completely suppressed (3 animals). The mammary glands showed stimulation and the uteri were enlarged. At the 1.0 mg. level the ovaries appeared unaffected, but at 2.0 mg. the corpora lutea were enlarged. The hypophyses showed degranulation of the basophiles.

Androstane-dione at the 1.0 mg. daily level in 2 rats completely and in 2 partially suppressed the estrous cycles. At a 2 mg. level complete suppression occurred in 2 animals. The ovaries of the animals whose cycles were inhibited showed fairly large corpora lutea. The ovaries of the remaining animals were either unaffected or slightly subnormal in size. Mammary glands and uteri showed erratic responses in this series. Pituitaries showed basophilic degranulation.

Androstene-dione at a 1.0 mg. daily level only partially suppressed the cycles of 2 animals. At the 2.0 mg. level suppression was complete in these same animals. The ovaries were definitely subnormal in size. Mammary glands and uteri were definitely stimulated.

Trans-androstene-diol at a 1.0 mg. level in 2 animals failed to influence the cycles or to significantly affect the ovaries, mammary glands or uteri.

Cis-androstene-diol in 2 rats at the 1.0 mg. daily level induced markedly prolonged periods of estrus. Thus in one animal the vaginal smear during the 22-day experimental period was of the estrous type for 16 days and in the other for 17 days. The ovaries appeared normal, the mammary glands were not stimulated, and the uteri were of the estrous type.

The tendency shown by cis-androstene-dione to induce continued estrus was even more apparent for dehydroandrosterone. The latter when injected at the 1.0 mg. daily level in normal rats resulted in estrous type smears for 16 of 18 days, 12 of 20 days, 19 of 22 days, and 21 of 22 days, respectively. The ovaries were small, the mammary glands were not stimulated, and the uteri were large.

The amazing effect of dehydroandrosterone in the adult normal female led us to administer this treatment to 4 hypophysectomized females. To our surprise, particularly in view of its failure to change the vaginal picture in the spayed rat, the hypophysectomized female reacted in a manner even more striking than the normal female. Estrous type smears were shown for 21 of 25 days, 22 of 22 days, 22 of 22 days, and 12 of 14 days, respectively. The ovaries were only slightly decreased, the mammary glands were not stimulated, and the uteri were enlarged.

All animals showed a marked enlargement of the preputial glands and in many instances a striking enlargement of the clitoris.

The marked tendency of cis-androstene-diol and dehydroandrosterone to produce periods of prolonged estrus in normal female rats may be due to their conversion by the ovary to an estrogenic substance. If this is true it seems apparent that ovarian function is not entirely suppressed by hypophysectomy since dehydroandrosterone shows the same effect in the hypophysectomized female rat.

9413 P

Maintenance of Spermatogenesis in Testis of the Hypophysectomized Rat with Sterol Derivatives.*

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From the Department of Anatomy, Wayne University, College of Medicine.

Walsh, Cuyler and McCullagh[1] reported that the administration of urine concentrates containing male sex hormone would prevent for as long as 20 days the degenerative changes which occur in the testes of hypophysectomized rats. These observations were confirmed and extended by Nelson and Gallagher[2] and Nelson,[3] who were able to show that spermatogenesis could be maintained for at least 60 days after hypophysectomy, and that such males were able

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* This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Physiol.*, 1934, **107**, 508.

[2] Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

[3] Nelson, W. O., *Anat. Rec.*, 1936, **67**, 110 (suppl.).

TABLE I.

Hypophysectomized Males Treated with Androgenic Hormones. Weights are given as averages.

No. of rats	Body Wt.		Treatment Hormone (daily dose) mg.	Organ weights (gm.)				Sperm motility
	First	Last		Testes	Seminal vesicles (full)	Seminal vesicles (empty)	Prostate	
			Testosterone					
3	229	195	2	1.287	2.982	.877	.733	++++
4	275	232	1	1.243	1.815	.651	.472	++++
4	234	208	0.5	1.127	1.708	.448	.398	+++
			Androsterone					
3	272	230	2.5	2.214	1.609	.529	.483	++++
3	281	237	1.5	1.882	1.159	.436	.365	++++
3	238	197	1.0	1.785	.958	.363	.277	+++
2	226	185	0.5	1.963	.498	.355	.220	+++
			Dehydroandrosterone					
3	245	210	3.0	2.120	1.145	.443	.454	++++
2	242	212	2.0	2.019	.859	.335	.359	++++
3	239	210	1.0	1.789	.627	.295	.276	++++
2	242	213	0.5	1.317	—	.163	.075	++
			Androstanedione					
5	276	219	1.5	2.379	1.529	.442	.517	++++
2	244	197	1.0	2.117	1.297	.375	.415	++++
3	248	207	0.5	2.141	.350	.206	.170	++++
2	237	208	0.25	1.221	.209	.175	.137	++
1	290	247	0.125	.899	—	.139	.073	No motility
			Androstenedione					
2	228	177	1.0	2.016	1.241	.460	.515	++++
			Androstenediol (cis)					
2	239	196	1.0	1.790	.497	.312	.215	++++
			Androstenediol (trans)					
2	225	185	1.0	.750	.142	.113	.084	No motility
			Oestrone					
5	258	174	1000 I.U.	.329	—	.074	.063	No sperm
			Oestrone, 1000 I.U., and Androstanedione					
5	247	183	1.0	1.909	1.183	.349	.373	++++
12	278	217	Controls	.527	—	.083	.054	No sperm
21	267	—	Normals	2.432	.963	.281	.295	++++

to sire normal litters. It was shown, however, that the interstitial cells are not stimulated and that male hormone preparations failed to reinitiate spermatogenesis in rats which had been hypophysectomized for several weeks. Nelson[3-4] reported that 4 synthetic androgenic substances, viz., testosterone, androsterone, dehydroisoandrosterone, and androstanedione would maintain spermatogenesis. This report presents further evidence on these and 3 other substances, viz., androstenedione, cis-androstenediol and trans-androstenediol.

In the experiments to be reported here the administration of the synthetic androgens† was begun on the second day after hypophysectomy and continued for 20 days. Table I presents the pertinent data concerning these experiments.

It will be noted that testosterone, the most potent known androgenic substance, is the least effective, with the exception of trans-androstenediol which is very weak, of all the androgenic substances in maintaining spermatogenesis. Dehydroisoandrosterone and cis-androstenediol, relatively weak androgens, are as active as the more potent, from an androgenic standpoint, androsterone. Androstanedione and androstenedione have about the same androgenic activity as androsterone, but are superior in their capacity to maintain the testes.

It seems apparent that the capacity of these substances to maintain spermatogenesis is unrelated to their androgenic activity as based on either the capon comb or rat sex-accessory tests. This is of considerable importance in view of the fact that Hamilton[5] and Cutuly, McCullagh and Cutuly[6] have recently expressed the opinion that the effect of androgenic substance on the testis of the hypophysectomized rat is due to its action in maintaining a normal scrotum. While there can be little doubt that androgenic hormone does have a stimulating effect on the scrotum we are convinced that this action plays a relatively minor rôle in the phenomenon. In animals receiving testosterone the scrotum is very large and pendant, yet the testes are markedly regressed and spermatogenesis, while in progress, is only moderately well maintained. In animals receiving

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[4] Nelson, W. O., *Anat. Rec.*, 1937, **67**, 36 (suppl.).

† The writers would like to express their appreciation to Dr. T. F. Gallagher (of Berlin), Dr. F. C. Koch (of Chicago), and to Dr. Erwin Schwenk (of the Schering Corporation) for the various androgens; to Dr. Oliver Kamm and Dr. O. A. McGinty (of Parke, Davis & Co.) for the oestrogenic hormone.

[5] Hamilton, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 386.

[6] Cutuly, E., McCullagh, D. R., and Cutuly, E. C., *Am. J. Physiol.*, 1937, **119**, 121.

androstane-dione at the 0.5 mg. level the scrota are definitely less well maintained, yet the testes are almost normal in size and spermatogenesis is well maintained.

Summary. Various crystalline androgens have been shown to maintain spermatogenesis in the hypophysectomized rat if treatment is begun soon after ablation of the hypophysis. They are not effective in re-initiating spermatogenesis. The capacity of these substances to maintain the testis appears to be unrelated to their male hormone activity.

9414 P

Effect of Oestrone on Hypophyses and Reproductive Organs of Thyroidectomized Rats.

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From the Departments of Anatomy, Wayne and Yale Universities.

Kojima,[1] Severinghaus, Smelser and Clark,[2] and Zeckwer[3, 4] have studied the hypophyses of thyroidectomized rats. Their reports agree that the basophiles increase in number and size and that the reaction is similar to that which occurs following gonadectomy. Zeckwer[4] considers that the reaction is sufficiently different to suggest the possibility that the basophiles are of 2 distinct types, *viz.,* those which react to gonadectomy and those which react to thyroidectomy. She further suggests that these 2 basophilic types are concerned in the production, respectively, of the gonadotropic and the thyrotropic hormones. Zeckwer also reports that the acidophiles are markedly decreased. The findings of Severinghaus, *et al.,* are in essential agreement as they find extensive acidophilic degranulation. Cells which their technique would show to be recently degranulated would be classified as chromophobes with the technique used by Zeckwer.

Zeckwer's suggestion that the cells which react to thyroidectomy are a separate type from those which react to gonadectomy suggested the treatment of a series of thyroidectomized rats with oestrone. This substance will prevent or correct the changes which occur in the basophiles following castration.

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[1] Kojima, M., *Quart. J. Exp. Physiol.*, 1917, **11**, 319.

[2] Severinghaus, A. E., Smelser, G. K., and Clark, H. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1127.

[3] Zeckwer, I. T., Davison, L. W., Keller, T. B., and Livingood, C. S., *Am. J. Med. Sci.*, 1935, **190**, 145.

[4] Zeckwer, I. T., *Am. J. Physiol.*, 1936, **116**, 166 (suppl.).

Twenty-five adult female and 12 adult male rats were thyroidectomized. Of these 8 females and 4 males were also gonadectomized. At the conclusion of the experimental period the hypophyses, gonads, adrenals, and accessory reproductive organs including the mammary glands were recovered for histological study. The techniques employed by both Severinghaus and Zeckwer were employed in representative instances. The entire thyroid region was sectioned serially and in every instance was found to be free of residual thyroid tissue.

Five T-animals* and 3 T-C-animals† were sacrificed on the 22nd day after thyroidectomy, 5 T- and 3 T-C-animals on the 42nd day, and 2 T-animals on the 122nd day. Five T- and 6 T-C-animals were sacrificed at 42 days, having received 40 R.U. of oestrone daily during the last 20 days. Five T-animals were treated during the entire 42-day period and 3 T-animals were sacrificed at 122 days, having received treatment during the last 30 days.

The hypophyses of the untreated animals showed the changes which have been described by others. It was possible to distinguish, as Zeckwer has indicated, between "thyroidectomy" and "castration" changes in the basophiles. However, the injection of oestrone in every instance completely or almost completely prevented, or in the cases of delayed treatment, corrected both types of changes. A marked decrease in the number of granulated acidophiles was present in all animals and was apparently unaffected by oestrone-treatment.

The effect of oestrone upon the ovaries of the females thyroidectomized for 42 days and injected the last 20 days was the same as in the normal female, *i. e.,* large corpora lutea were present. In the animals injected during the entire 42-day period or during the last 30 days of the 122-day period the ovaries were very small and showed no corpora lutea. The mammary glands of all animals receiving oestrone showed marked development. This is in agreement with the earlier reports (Nelson[5] and Nelson and Tobin[6]) that oestrone alone will induce marked mammary gland growth in the rat and that mammary development may occur in the absence of the thyroid.

In the untreated thyroidectomized males the reproductive organs were normal in weight and miscroscopic appearance. The reproductive organs of the males receiving oestrone showed the same marked damage that occurs in the intact male treated in a similar fashion. The testes were markedly atrophied and the accessories were very small.

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* T animals refers to thyroidectomized animals.

† T-C animals refers to thyroidectomized-castrated animals.

[5] Nelson, W. O., *Anat. Rec.,* 1935, **64**, 52 (suppl.).

[6] Nelson, W. O., and Tobin, C. E., *Endocrinology,* 1937, in press.

The effectiveness of oestrone in preventing and correcting the changes which occur in the hypophysis following thyroidectomy is similar to its action on the changes that follow castration and appears to be evidence against the idea that the basophiles which react to the 2 operations represent 2 different cell types. It seems probable that there is only one type of basophilic cell in the anterior hypophysis. This cell in different phases of its secretory cycle may react to castration in one fashion and to thyroidectomy in a slightly different manner.

9415 P

Further Experiments on the Grafted Eye and the Regeneration of the Lens in Amphibians.

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These studies are another part of a series of investigations which have already been reported[1-4] on the grafted vertebrate eye. The present one is based upon results obtained in 262 operated eyes in an adult salamander, *Triturus viridescens.* In 110 cases the eye was excised and reimplanted in its orbit, *i. e.,* replaced in its normal environment. In 90 cases the lens was removed through an incision made in the cornea. In 62 cases the main blood vessels leading to the eye were severed either along with or without the optic nerve. In a majority of animals the eyes and lenses were measured and dissected in the fresh state.

When the eye is reimplanted there is some reduction in its size followed by a degree of recovery. Careful measurements of the fresh eye show that up to 365 days there is no complete recovery.

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[1] Stone, L. S., and Ussher, N. T., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 213.

[2] Stone, L. S., *J. Exp. Zool.,* 1930, **55**, 193.

[3] Stone, L. S., and Cole, C. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 176.

[4] Stone, L. S., Zaur, I. S., and Farthing, T. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1082.

Cases vary from 85% to 98% of attaining their normal size during this time. In the specimens from which the lens was removed there was also a slight decrease in the size of the eye following operation. This, however, proved to be only temporary, for there was complete recovery by the 120th day.

When the eye is reimplanted the lens disintegrates as shown in previous experiments on adult amphibians (op. cit.). The new lens in this type of experiment begins to develop from the dorsal part of the pupillary margin of the iris from 18 to 20 days after operation. At the end of 365 days the size of the regenerated lens varies from 72% to 92% of its original measurements. In size the degree of recovery is approximately the same as that of the bulb as a whole.

In those cases where the lens was eliminated by removing it through an incision in the cornea the new lens originated in the same manner as in the reimplanted eye. This supports recent findings of Sato.[5] However, the first appearance of the regenerating lens was earlier than in the grafted eye, for it was first seen as early as the tenth day. At the end of 200 days the new lens is varied from 75% to 95% of the normal size. From a study of the ratio of lens and eye sizes there is no indication that the regenerated lenses would reach normal size if left for a much longer time.

The cutting of the optic nerve with its accompanying blood vessels leading to the eye does not stop the circulating blood which one can see moving in the iris. Also the circulation in the eye is not eliminated by merely cutting all the blood vessels leading from the walls of the orbit to the eye and leaving the optic nerve intact. In either type of experiment the presence of a small amount, but continuous supply, of circulating blood is sufficient to prevent the disintegration of the lens and retina. All vessels and the optic nerve must be severed in order to produce the same effect as in the case of the grafted adult eye.

In those cases where the optic nerve was severed the regeneration of the nerve was rapid. Three specimens selected for tests for return of vision in the operated eye showed visual function 63, 65 and 70 days respectively, after operation. This is about the time one finds it in the grafted eye of this species. The technique for visual tests was the same as that already described in detail.[2]

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[5] Sato, T., *Arch. f. Entwmech. d. Org.*, 1933, **130**, 20.

9416 P

Induction of Pseudopregnancy in Rat by Silver Nitrate on Nasal Mucosa.

Samuel Rosen and M. C. Shelesnyak. (Introduced by Robert T. Frank.) (With technical assistance of Philip Rosenberg and Helen v. Rosen.)

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Physiologic relationship of nasal and genital regions has been described chiefly by Fliess[1] and Brettauer.[2] Recent studies have been made by Collip and his collaborators.[3] Suggestive clinical observations by one of us (Rosen) stimulated a series of laboratory experiments in an effort to establish evidence of a specific naso-genital mechanism. Results reported herein are taken from a large and varied group of experiments.

Adult female albino rats, from an inbred colony, were used. They were isolated from males, fed a diet of hospital table scraps supplemented with greens, Pablum, powdered milk, and cod liver oil. The regularity and the nature of the sexual cycles of all the stock females were determined from vaginal smears. Only rats with regular 4 or 5 day cycles were used. After the observation of 4 to 6 consecutive regular cycles, treatment consisting in the application of silver nitrate solution to the mucosa of the conchae was started. The silver nitrate solution was applied in one group with a fine cotton applicator; in a second group, by instillation of 0.05 cc. $AgNo_3$ using a tuberculin syringe. The time of treatment was: (a) during the proestrus and estrus of 2 consecutive cycles, a total of 6 days with interruption by 2 rest days, and (b) during diestrus, proestrus, estrus and diestrus, a total of 6 days application. This second type of treatment was more generally used. Certain animals received 10%, others 20% and still others 50% (gm., %) solutions during entire treatment period; others received progressively increased doses of 10, 20, 50% for 2 days each. Two control studies were made by (a) using NaCl (0.9%) and (b) applying silver solutions to the buccal mucosa. All cases, experimental and control, were treated under light ether anesthesia.

A group of the animals which experienced a post-treatment prolongation of the diestrous phase was autopsied during diestrus and

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[1] Fliess, H., 1897, Leipzig and Wien.

[2] Brettauer, J., *Am. J. Obs. and Dis. Women and Children*, 1911, **64**, No. 2.

[3] Mortimer, H., Wright, R. P., Collip, J. B., *Canadian M. J.*, 1936, **35**, 503.

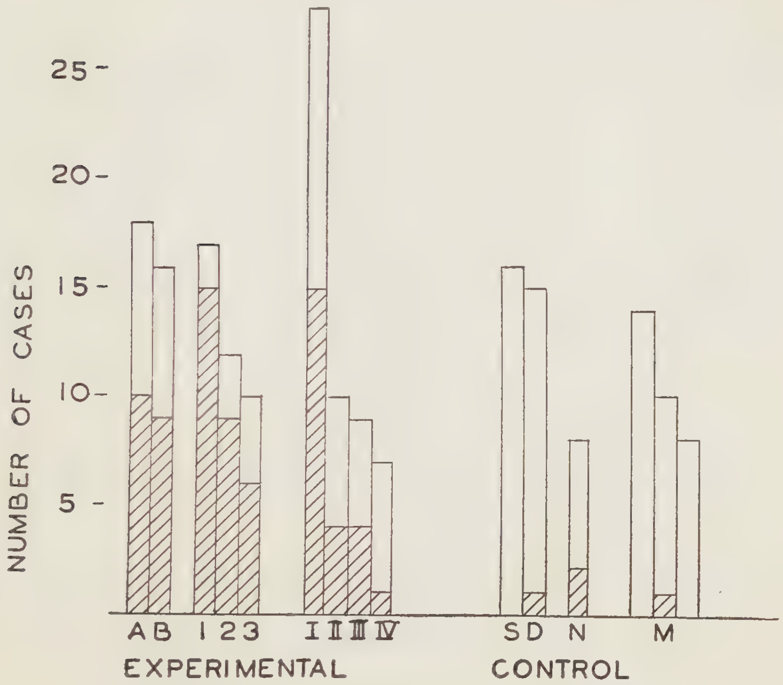

Fig. 1.

Graph shows number of cases in each series and positive responses (cross-hatched).

Experimental—A, B = 2 groups receiving 10, 20, 50% progressively, 2 days each, with cotton applicator.

1, 2, 3 = 3 groups treated as A, B but with instillation by catheter.

I, II, III = 50, 50; 10, 20; 10, 50% respectively, with cotton applicator, on two consecutive estrus periods.

IV = 10% with applicator for 6 consecutive days.

Control—S, D = Nasal application with dry catheter for 6, 4 days.

N = Nasal application 0.9 NaCl, for 6 days.

M = Buccal application of 10, 20, 50% progressively, 2 days each concentration.

the ovaries examined; a second group was operated on the 4th or 5th day of leucocyte phase and the uteri were traumatized with silk thread loops (longitudinal—Shelesnyak[4]) to provoke deciduoma formation.

Of 186 animals included in this study, 127 were treated intranasally with silver nitrate. The weighted average length of 1400 cycles was 4.1 days. Prolonged cycles ranged from 8 to 21 days. Of the 127 experimental animals, 69 (or 54%) cases experienced post-treatment prolongation of leucocyte phase. One technique of treatment induced 30 positive reactions of 59 trials (77%). Three cases evinced a decidual reaction in the traumatized uterus. Of the

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[4] Shelesnyak, M. C., *Am. J. Physiol.*, 1933, **104**, 693.

69 controls 4 cases of prolongation occurred; 3 of them 8-day cycles and one a 13-day. Three of these 4 prolonged cycles were in cases which received nasal manipulation but no silver salt.

The findings establish the existence of a naso-genital relationship whereby nasal stimulation elicits an ovarian response, a diestrual prolongation. On the basis of luteal ovaries and decidual reactions in traumatized uteri, this prolongation must be considered pseudopregnancy.

The authors are grateful for the helpful guidance of Dr. Robert T. Frank.

9417

Is Antibody-Globulin "Denatured" by Its Combination with Antigen?

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It has frequently been stated that antibody becomes "denatured" when combined with its antigenic or haptenic counterpart. "Denatured" is one of our slippery words which has a loose rather than a definitive signification. Perhaps "lessened solubility" most commonly expresses what is meant by denaturation. Shibley[1] has recorded the remarkably identical electrophoretic behavior of thermally denatured normal globulin and of bacteria thoroughly coated with antibody-globulin. But, that antibody-globulin has not really lost its natural (and very labile) qualities as a result of its combination with antigen, is shown by the facts that dissociated antibody retains its specific combining characteristics, and immune sera produced by injecting antibody-antigen complexes not only reflect the species-specificity of the serum,[2] but also the particular features of different kinds of antibody, *e. g.*, that of antipneumococcal serum is antigenically somewhat different from diphtheric antitoxin[3]—each having been combined with its antigen before injection into the antibody-producing animal.

To compare, in another way, the properties of specific globulin-antigen compounds and thermally denatured globulin we have mixed an opalescent solution of the latter (normal rabbit's serum, diluted with 3 volumes of water, steamed for 15 minutes, and centrifuged) with specific precipitative systems (hemocyanin and ovalbumin) and have found that the amounts of precipitated nitrogen were no greater than those produced in mixtures not containing the heated normal serum whether in the zone of equivalence, of excessive antigen, or of excessive antibody. In these respective zones the comparative figures, averages from duplicate analyses, were 0.512 and 0.489; 0.517 and 0.509; 0.402 and 0.408 mg. N in the *Fulgur carica* hemocyanin-system; 0.103 and 0.110; 0.106 and 0.117; 0.172 and 0.181 mg. N in the *Limulus polyphemus*-system. Mixtures of ovalbumin and its antiserum (rabbit) were made only in the zone of equivalence and of excessive antibody. The nitrogens in the precipitates were 0.743 and 0.749; 0.498 and 0.502 mg.

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[1] Shibley, G. S., *J. Exp. Med.*, 1929, **50**, 825.

[2] Braun, H., *Z. f. Immunitätsf.*, 1909, **3**, 53.

[3] Ando, K., Kee, R., and Komiyama, T., *J. Immunol.*, 1937, **32**, 181.

Thus, the prominent cohesive property of specifically combined antibody-globulin is not developed by thermally denatured normal globulin, although, in Shibley's experiments, the ζ-potentials of the two forms were identical. This negative evidence is reported because it slightly narrows the field wherein we must search for an answer to the question: What makes combined antibody sticky?

9418 P

Occurrence of Birefringence in the Fertilized Egg of the Sea Urchin.

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In preliminary experiments it was observed that, after fertilization, the eggs of *Strongylocentrotus purpuratus* showed, near the periphery, light and dark phases when rotated between crossed Nicols. The effect, however, was partially masked by the opaque granules of the cytoplasm. In order to eliminate this difficulty the unfertilized ova were centrifuged at approximately 60,000 gravities for 3 minutes, flotation of the eggs being insured by the addition of sufficient M/1 solution of sucrose to the sea water in the tubes. After centrifugation the eggs were transferred to sea water and microscopic preparations made in the usual way. The eggs appeared elliptical in shape, with the nucleus at the centripetal end and the opaque granules at the opposite pole occupying about ⅓ of the egg. The remaining ⅔ of the egg was transparent and showed no birefringent effects with polarized light. Within 3 minutes after fertilization, however, when the membrane was forming and afterward, the clear cytoplasm gave evidence of birefringence so definite that the angle between light and dark phases could be measured. In all 35 measurements of the angle were made and these yield a mean value of 31°, with extremes of 24° and 37°, the intermediate values being consistently distributed on a probability curve. Now the optical section of the egg is something less than 72μ, and since such a minute quantity of polarizing molecules (*e. g.*, sugar) in solution would not be sufficient to rotate the beam of polarized light, it is necessary to assume that the rotation is due to a regular grouping of molecules to form a multimolecular lattice with birefringent properties similar to those of a crystal, a starch grain, a filament of silk, a muscle fibril. Fertilization, therefore, causes an arrangement in regular pattern of certain molecules or micels of the cytoplasm. It is obvious that with crossed Nicols there would be, with an anisotropic crystal, 4 dark and 4 light phases for a complete rotation of the specimen, whereas we have obtained 6. The solution of this problem probably lies in the same direction as that of the peculiar birefringence of the starch grain. The effect which we have obtained may be related to the chemical change reported by Mirsky,[1] who found that the percentage of soluble protein in the egg of the sea urchin diminishes and that of the insoluble protein increases as the result of fertilization. A relation of the effect under consideration to the migration of the nucleus after fertilization is also suggested, since it has been shown that the nucleus is probably pulled into position by contractile structures in the cytoplasm.[2]

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[1] Mirsky, A. E., *Science*, 1936, **84**, 333.

[2] Moore, A. R., *Protoplasma*, 1937, **27**, 544.

9419 P

Effect of Digitalis Administration on Experimental Hypertrophy.*

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The experimental studies that have been cited in support[1] of the contention that digitalis administration inhibits or retards the development of cardiac hypertrophy under conditions which usually bring it about, are not conclusive. Cloetta's[2] conclusions are open to criticism, because too small a number of experimental animals were used and because the doses of digitalis administered to his animals were much greater than humans could possibly tolerate.

It was therefore considered desirable to repeat the studies obviating as nearly as possible these criticisms. In order to accomplish these ends some 200 rabbits have been operated upon with the carotid valvulotome for the production of experimental aortic regurgitation. A total of 60 animals with aortic regurgitation survived for 3 weeks or more and approximately two-thirds of the rabbits were given by intramuscular injection, 0.1 and 0.3 cat unit per kilo of digalen, digifoline, and digiglusin twice weekly. This dosage was calculated to maintain digitalization.

Of 17 rabbits in which only small lesions, merely the perforation of one valve cusp, had been accomplished, 11 had been digitalized and 6 had received no drug. Only slight hypertrophy was established in the hearts of these animals. No difference was noted in the untreated and treated rabbits.

In 43 rabbits one or more cusps were destroyed and considerable cardiac hypertrophy had been produced. Digitalization was carried out in 26 of these animals with aortic regurgitation for 30 to 147 days with an average of 94 days. The 17 non-treated rabbits with experimental aortic regurgitation were sacrificed after 38 to 104 days, an average of 92 days. The details of the findings are shown in the diagram. The normal HW/BW was previously established to be 0.197.[3]

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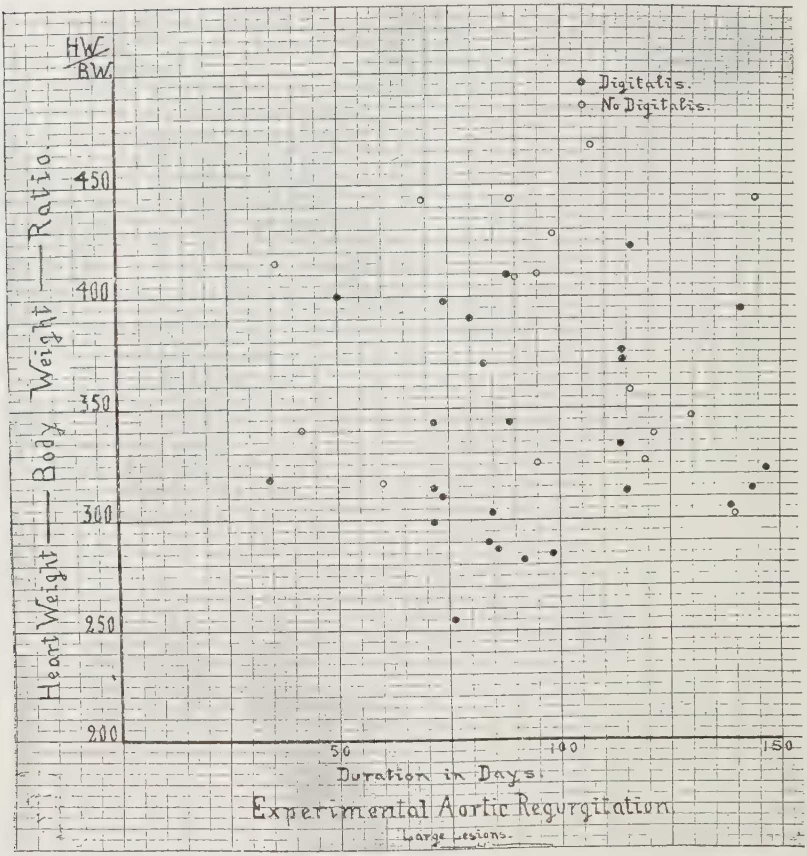
* Supported by Grant 349 and 392 from the Committee of Scientific Research of the American Medical Association.

[1] Christian, Henry A., *J. A. M. A.*, 1933, **100**, 789.

[2] Cloetta, Max, *Arch. f. exp. Path. u. Pharmakol.*, 1908, **59**, 209.

[3] Herrmann, G., Decherd, G., Erhard, P., and Schwab, E. H., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 409.



The difference between the mean values of HW/BW ratios for the non-digitalized and the digitalized groups is statistically significant according to the method of Fisher, section 24, 1. The probability of the chance occurrence of this difference is less than 0.01. Although our results are not nearly so striking as those of Cloetta, the evidence seems to indicate that digitalis does have some restraining effect on the development of cardiac hypertrophy. Nevertheless we feel that a still larger series of animals should be similarly studied.

9420 P

Bactericidal Action of Pectin.

Edith Haynes, Charles A. Tompkins,* Grace Washburn and Matthew Winters. (Introduced by O. M. Helmer.)

From the Indiana University Medical Center.

The possibility of pectin having an effect on bacteria was considered in connection with a study of the therapeutic action of a pectin-agar, dextrimaltose mixture in the treatment of diarrheas of infants.[1] In order to test this possibility the following experiment was carried out.

Two percent pectin† added to heart-infusion broth (Difco) at pH 7.6, lowered the pH to 5.0-5.4. The medium was autoclaved at 15 pounds for 20 minutes and then transferred to sterile tubes in 10 cc. quantities. A tube of this medium was inoculated with a 4 mm. loopful of an 18-24-hour culture of *Esch. coli* and was shaken 25 times to insure thorough distribution. Dilution plates were made immediately, then after 24 hours and again after 48 hours of incubation at 37°C. A control medium without pectin was prepared in the same way and uninoculated controls were used to check the technic. It was found that the broth containing the pectin was either sterile after 48 hours or the number of organisms had decreased at least 98% while the bacteria had multiplied in the control cultures. This experiment was repeated using the same medium adjusted to pH 5.5, 6.0, 6.5 and 7.0. Illustrations of the results of these tests are shown in Table I. It has been shown that pectin is readily altered by alkali[2] which would explain the loss of the inhibitory action above pH 5.5. It could not be concluded from this work that pectin would decrease the bacterial count in the gastro-intestinal tract, particularly in the lower bowel where the reaction is normally above pH 5.5.

Some preliminary work has been done on the treatment of wounds with aqueous solutions of pectin with very satisfactory results. These wounds have included those of osteomyelitis and other deep wounds as well as superficial wounds, some of which had not responded well to accepted methods of treatment. An aqueous solution of pectin seems to be non-irritating and apparently stimulates growth of gran-

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* Mead Johnson Research Fellow in Pediatrics.

[1] Winters, M., and Tompkins, C. A., *Am. J. Dis. of Child.*, 1936, **52**, 259.

† Kindly furnished by Sardik Laboratories, New York.

[2] Myers, P. B., and Baker, G. L., *Univ. of Del. Agri. Exp. Station Bull.*, 1934, 187.

TABLE I.
Effect of 2% Pectin on Growth of *Esch. coli* in Heart Infusion Broth.

	pH	Original Count per cc.	Count after 24 hr. per cc.	Count after 48 hr. per cc.	After 48 hr.	
					% Increase	% Decrease
Pectin	5.0	185,000	375	0		100
Control	5.0	251,000	287,000,000	935,000,000	372,410	
Pectin	5.2	147,000	3,700	69		99.9
Control	5.2	37,000	725,000,000	1,337,000,000	3,613,414	
Pectin	5.4	71,000	500	43		99.9
Control	5.4	78,000	31,000,000	75,000,000	96,053	
Pectin	5.5	90,000	4,967	1		99.9
Control	5.5	323,000	513,333,000	2,030,000,000	625,287	
Pectin	6.0	97,000	311,000	390,000,000	401,962	
Control	6.0	250,000	677,000,000	1,103,000,000	441,100	
Pectin	6.5	171,000	310,000,000	720,000,000	420,952	
Control	6.5	167,000	525,000,000	1,117,000,000	668,763	
Pectin	7.0	144,000	286,000,000	315,000,000	218,650	
Control	7.0	129,000	506,000,000	745,000,000	577,420	

ulative tissue. In cultural studies, it was found that as the wounds healed there was a marked decrease or complete disappearance of streptococci and a more gradual diminution of staphylococci. It is possible that the action of pectin on bacteria is one of the factors in the clinical success of this treatment.

9421 P

Effects of Progesterone on the Female Genital Tract after Castration Atrophy.*

FREDERICK L. HISAW, R. O. GREEP AND H. L. FEVOLD.
From the Biological Laboratories, Harvard University.

The effects of oestrin-free progestin on the atrophic endometria of 2 castrated monkeys were described by one of us.[1] These animals had been castrated for 37 days and were given 4 RbU of oestrin-free corpus luteum extract daily for 10 days. The endometria of both monkeys showed unquestionable premenstrual development. Although these preparations did not produce cornification of the vaginas of castrated rats it could not be stated with certainty that they were entirely free of oestrin and that the observed effects were due only to the action of progestin. We wish here to present results of similar experiments using synthetic progesterone.†

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* Aided by a grant from the Rockefeller Foundation.

[1] Hisaw, F. L., *Am. J. Obst. and Gyn.*, 1935, **29**, 638.

† The synthetic progesterone was furnished by the Schering Corporation.

A young adult monkey that had been castrated for 242 days was given 4 mg. of progesterone daily for 18 days, a vaginal biopsy was taken on the eighth day, a thread was placed through the uterus on the eleventh day and the animal was killed on the nineteenth. Nucleated epithelial cells appeared in the vaginal smear and were present in considerable numbers by the eighth day, after which they gradually decreased and were almost absent at the conclusion of the experiment. The face showed coloration by the seventh day and the pale sexual skin developed and maintained a deep red color. The uterus, at laparotomy on the eleventh day, measured 11 by 9 mm. at its greatest diameters and at autopsy 17 by 13.5 mm.

This treatment produced a fully developed premenstrual endometrium and epithelial proliferations[1] characteristic of an implantation site. The cervical glands showed active secretion though the epithelial cells in most places were not as tall as those seen following injections of oestrin. The vaginal mucosa, though thin, showed some stimulation both at the time of biopsy on the eighth day and at autopsy.

Experiments similar to those described for monkeys were also carried out on castrated rabbits. A rabbit that had been castrated one year was given 6 RbU of oestrin-free progestin distributed equally over 5 days and killed on the sixth. Another, 8 weeks after castration received one RbU of progesterone daily for 7 days and was killed on the eighth. Two rabbits, 12 days after castration, were given 0.25 and 1.0 mg. of progesterone daily for 7 days and killed on the eighth. The uteri of all these animals showed growth, mitoses and progestational modification of the endometrium. A +++ to ++++ reaction was obtained in all these rabbits except the one receiving ¼ mg. progesterone daily which showed about +.

The effects of synthetic progesterone were tested on 4 adult female rats, 2 of which had been castrated for 150 days and 2 for 168 days. One of each pair was hysterectomized just previous to the beginning of the experiment for the purpose of determining whether or not the uterus had an influence on possible vaginal changes. The effects on the vagina were followed by vaginal smears and biopsies. Each animal was given 1.0 mg. of progesterone daily for 6 days and killed on the seventh. This treatment produced changes in both the uterus and the vagina. The uteri of the experimental animals were slightly larger than those of the castrated controls and the uterine and glandular epithelium showed an increase in thickness and occasional mitotic figures. A few nucleated epithelial cells usually appeared in the vaginal smears of both the hysterectomized and castrated animals by the fourth or fifth day of treatment and biopsies and examination at autopsy showed a slight though definite thickening of the vaginal mucosa associated with some mitotic activity and scattered glycogen granules.

Although synthetic progesterone was used in most of these experiments, the results suggest the presence of an oestrogenic substance. An attempt to check this possibility was made by collecting the urine before and during the treatment with progesterone and analyzing it for oestrin. The urine of 2 monkeys and 3 rabbits that were given from 0.25 to 2.0 mg. of progesterone daily was extracted‡ and tested on ovariectomized rats. In no instance were we able to obtain an effect on the vaginal mucosa.

It seems quite probable that these effects are due to the direct action of relatively large doses of progesterone. It has been found previously that one RbU of progestin distributed over 5 days has little or no effect on the uterus of the rabbit after complete castration atrophy,[2] while Lloyd[3] has found that 0.5 mg. progesterone daily for 3 days stimulates mitotic activity. It is also of interest to note that certain of the androgens produce progestational proliferation of the endometrium of rabbits[4] and growth of the mammary glands of rats[5] thus displaying the physiological properties of both progesterone and oestrin. These observations show that these chemically related hormones are adapted for producing their specific effects when present in small amounts yet they have, to a smaller degree, certain physiological characteristics in common which can be demonstrated under favorable circumstances by increasing the dosage.

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‡ These urines were extracted by Dr. Pincus and associates by methods developed in their laboratory.

[2] Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1930, **92**, 574.

[3] Lloyd, C. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 190.

[4] Klein, M., and Parkes, A. S., *Proc. Roy. Soc.*, 1937, **121**, 574.

[5] Selye, H., McEuen, C. S., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 201.

9422

Effects upon Blood Amylase of Variations in Thyroid Activity.

Willard Bartlett, Jr. (Introduced by M. Somogyi.)
From the Laboratory, Jewish Hospital, St. Louis.

The sensitive method of Somogyi for the quantitative determination of blood amylase[1] permits the estimation of very low quantities of the enzyme. This quality of the method has led to the observation that low levels of blood amylase are found as a rule whenever liver functions are impaired.[1] Low amylase values were frequently observed in cases where dysfunction of the liver was of a moderate degree and could not be proved by the customary clinical methods employed for estimating liver function. It is the view of Somogyi and his coworkers, based on a very large experience, that amylase derives largely from the liver, hence the level of amylase in the blood is considered to be an important indication of liver function.

We have had the opportunity of doing occasional estimations of blood amylase since 1934 in the course of other work and the frequent finding of rather low values in patients with thyrotoxicosis, particularly before treatment is instituted, has seemed to warrant an investigation of serial determinations at varying levels of thyroid activity. This has been followed in two directions: 1. as thyroid activity increases with thyroid feeding in hypothyroidism; 2. as thyroid activity in hyperthyroidism is diminished by treatment with iodine and thyroidectomy.

The blood amylase for normal subjects will fall between values of 80 and 150 in about 90% of cases; approximately 5% give values down to 50 and a further 5% range from 150 to 200; the individual values remain remarkably constant for the healthy person. Before the serial study which forms the subject of this report was undertaken, the following isolated observations were made on patients with toxic goiter.

1. Untreated: 1/3/35, M.A.V., 60; 5/24/35, E.B., 30; 5/18/35, B.M., 66.
2. Treated (preoperative): 12/10/34, R.C., 72; A.H., 4/26/35, 108; 4/23/35, M.K., 75; 4/22/35, M.V., 86; 5/17/35, A.J., 54.
3. At discharge following operation: 12/17/34, L.S., 78; 4/29/35, M.V., 66; 6/1/35, B.M., 108; 6/6/35, A. J., 75.

In the tables which tabulate the results of the present study the column headed "D" records the value of the blood amylase, which term is preferred to the older one, diastase. Table I indicates that hypothyroidism or even myxedema may develop without abnormal

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[1] Somogyi, M., Proc. Soc. Exp. Biol. and Med., 1936, **32**, 538.

TABLE I.
Effect of Thyroid Feeding on Untreated Hypothyroidism.

Patient			Before				After			
Name	Age	Sex	Date	BMR	D.	daily dose	Date	BMR	D.	
E.S.	53,	♀	12/2/36	—24	168	gr. ii	3/2/37	—	133	much imp.
E.W.	42,	♀	1/7/37	—24	94	iss	2/18	—12	114	,, ,,
C.S.	41,	♀	1/26	—12	70	ii	3/17	—7	52	unchanged
E.T.	56,	♀	2/12	—8	84	ii	3/23	0	73	much imp.
F.D.	52,	♀	2/11	—12	160	ii	4/17	+14	88	*toxic* 4/17
R.T.	40,	♀	2/17	—24	114	i	4/20	—20	89	imp.
E.L.	45,	♀	3/2	—16	84	ii	4/20	—10	73	sl. imp.
Effect of { increasing dose of Thyroid in Hypothyroidism. decreasing										
F.H.	32,	♀	12/29/36	—12	145	i→ii	3/2/37	—12	123	imp.
E.B.	26,	♀	1/8/37	—2(?)	114	i→iss	3/16	—11	100	,,
H.W.	29,	♀	2/11	—10	123	i→ii	3/23	—9	94	,,
H.H.	54,	♀	2/18	—6	47	i→ii	4/20	+12(?)	47	,,
K.H.	47,	♀	1/14	—9	46	ii→i	3/4	—16	57	? toxic 1/14
L.D.	35,	♀	4/17	—2	46	ii→i	5/15	—9	55	toxic 4/17

blood amylase values. This must not be taken to mean that values above the normal range may not be found in a larger series of cases since only E.S. and E.W. had spontaneous hypothyroidism, the remainder of the cases being of postoperative hypothyroidism; as will be seen later, the amylase may rise only very slowly following operation and in Table III is recorded a value of 67, two months following operation, at a time when the patient had developed myxedema. Further study of spontaneous hypothyroidism and of very long-standing postoperative hypothyroidism may reveal grossly *elevated* values. The tendency of the blood amylase to fall, however, in response to thyroid medication is unmistakable, only one patient (E.W.) showing a rise. The fall is quite abrupt in F.D., who showed definite thyroid intoxication on 4/17. In the second portion of Table I, H.K. and L.D. show a slight rise after decrease

TABLE II.
Effect of Thyroid Feeding on Neurotic Patients with Questionable Hypothyroidism.

Patient			Before				After			
Name	Age	Sex	Date	BMR	D	daily dose	Date	BMR	D	
M.E.	35,	♀	12/3/36	—16	123	ii	1/15/37	—7	123	no imp.
A.L.	50,	♀	1/6/37	—25	100	iss	1/29	—19	73	,, ,,
H.R.	39,	♀	1/8	—20	80	ii	1/29	—4	80	,, ,,
B.H.	47,	♀	2/4	—11	73	i	3/17	—6	88	,, ,,
M.H.	32,	♀	3/25	—15	123	ii	5/8	—	64	sl. *toxic* 5/8
Effect of decreasing dose in same group.										
G.W.	33,	♀	12/23/36	—2	88	ii→0	1/25/37	—13	88	no change
M.W.	35,	♀	1/5/37	+6	133	ii→i	3/9	—6	100	*toxic* 1/5
M.S.	29,	♀	1/17	+6	50	ii→ss	3/6	—12	55	*toxic* 1/17

of thyroid dosage from 2 grains of desiccated thyroid substance to 1 grain, daily. Patient C.S. was not distinctly hypothyroid in any respect before thyroid feeding was started; she had a recurrence of palpable thyroid tissue some eighteen months after operation for toxic goiter and was given thyroid substance in the hope that further growth of her goiter would be prevented.

In Table II is recorded experience with a group of patients whose well-defined neuroses more or less closely simulated hypothyroidism. These patients were usually given only a short course of thyroid feeding as a therapeutic test (M.E. took thyroid only 3 weeks, as did H.R. and A.L.) and little or no change is seen in these individuals, but M.H., who became mildly thyrotoxic, shows a pronounced fall in amylase. An unexplained rise is seen in one case (B.H.) and an unexplained fall on decreasing the dosage in another (M.W.), although the latter patient was so highly erratic as to somewhat vitiate any conclusions; she also had several severe upper respiratory infections during the period of observation and any intercurrent infection may lower the blood amylase. The time element is of importance here, for in M.E., H.L. and G.W. no change in amylase is noticed over a period of a month or less although the basal metabolism is appreciably altered within this length of time.

TABLE III.
Thyrotoxicosis: Effect of Treatment.

Patient			Unstabilized			Stabilized				Post-operative		
N.	Age	Sex	Date	BMR	D	Date	BMR	D	Op.	Date	BMR	D
A.H.	46,	♀	12/8/36	+26	59	12/21/36	+14	53	12/22	2/5/37	—4	80
R.M.	61,	”	12/2		152				12/17 & 28	2/17	—2	230
M.G.	37,	”	12/3	+30	24	3/17/37	+25	15	3/18 & 26	4/17	—8	29
A.L.	38,	”	2/4/37	+27	38	2/16	+6	53	2/18	4/21*	—24	67
R.T.	40,	”				12/30/36	+10	75	1/2/37	2/17*	—24	114
M.V.	20,	”	2/5	+11	74	3/29/37	—21	100				
D.B.	42,	”	2/16	+20	123	4/21	+13	145				
A.F.	51,	♂				12/5/36	—1	80	12/10	12/31	—24	84
L.B.	39,	♀				1/8/37	+31	66	1/9	1/15	+7	70
P.B.	46,	”	2/23	+51	73	4/28	+26	94				

Table III indicates the occasionally very low values that are found in thyrotoxicosis and the tendency for the amylase to rise when important improvement is seen in the basal metabolism and in clinical status. There has not been any apparent difference in the behavior of the amylase in respect to the type of goiter with which the thyrotoxicosis was associated in this small series, solitary adenoma, multinodular goiter and diffuse hyperplastic goiter all being represented. The direction of change is the same for all types, once thyrotoxicosis is established; larger series of cases may reveal quantitative differences varying with the pathological classification. (In one patient, not included here, C.H., no activity whatever of the serum had occurred after 30 minutes of incubation; this indicates an almost complete lack of activity of the ferment.) Eight determinations were made before operation on patient M.G. over a period of three months during which she was highly refractory to treatment and seemed to be on the verge of thyroid crisis at times. The amylase values were exceedingly low, varying from 15 to 48 during these exacerbations and remissions of her condition. In the first five cases recorded in Table III, the postoperative observations have been made at a sufficiently remote period that the amylase has reached or surpassed the preoperative values, yet M.G. is still far below normal and, as has been noted, A.L. had actually developed myxedema though her diastase had come up only to the lower ranges of normal. R.T. had also developed myxedema though her preoperative figure was not so low as with M.G. and A.L. One would judge from this that the return of the blood amylase to normal lags behind the improvement in other respects, as judged by basal metabolism and the circulatory status. This is also indicated by the preoperative course of A.H. and A.L. and by the early postoperative observations on A.F. and L.B.

TABLE IV.
Behavior of Amylase in Early Post-op. Phase.

Patient			Pre-op.		Post-op.			
				Amylase				
Name	Age	Sex	BMR		8 hr.	24	48	5 d.
G.H.	60,	♂		50	48	53		
temp. F.					98.6	99		
E.J.	27,	♀		52	53	55		
temp.					99	98.8		
P.B.	46,	♀	+26	94	48	47	52	59
temp.					99	100.2	98.6	98.6
G. R.	36,	♀	+16	76	42	35	43	57
temp.					98.6	98.6	98.8	98.6
K.P.	22,	♀	+15	114	84	80		
temp.					99.4	102		
E.S.	47,	♀	+5	73	46	41	66	
temp.					99	99	99.6	

More striking evidence of the importance of the time element and further indication as to the behavior of blood amylase in thyrotoxicosis is furnished in Table IV, in which serial observations are recorded for the immediate postoperative phase, during the period in which an exacerbation of all signs of thyroid intoxication is produced by the operation itself. In thyrotoxic patients a thyroid crisis in miniature is produced during the first 48 hours after operation; on the other hand, little or no reaction is observed following operations on non-toxic goiters. The two patients in Table IV, G.H. and E.J., who were operated on for non-toxic, encapsulated adenoma of the thyroid, show no variation in the amylase during the immediately postoperative phase although their preoperative levels are low. Quite different is the reaction to operation of the other four patients, P.B., G.R., K.P., and E.S., who were distinctly thyrotoxic and were operated on for diffuse hyperplastic goiter after the usual preparatory treatment. They show an immediate, important fall in blood amylase in observations made 8 hours after operation; this low level is maintained for at least 48 hours and the preoperative level may not have been reached at the time of discharge from the hospital in 5 days. As shown in the table, this process may occur without significant febrile reaction.

There has long been reason to suspect that there was functional impairment of the liver in thyrotoxicosis, though no conclusive evidence as to its constancy or as to the mechanism involved has been available. The occasional jaundice seen in patients dying in thyroid crisis and autopsy findings indicative of hepatitis have not been clearly distinguishable from similar occurrences complicating other febrile forms of exitus; there is much further similarity between the clinical courses of patients in thyroid crisis and in the terminal stages of acute hepatitis in that both are marked by fever, delirium and coma and loss of vasomotor tone with fall in blood pressure. There is ample evidence in the German literature of the last 10 years that the blood lactate rises to abnormal levels and returns very slowly to the resting level after measured exertion in thyrotoxic patients and this has been attributed to improper resynthesis of lactic acid to glycogen on the part of the liver. Finally, the author[2] has reported that death occurs in postoperative thyroid crisis even though an afebrile course be maintained by the employment of a continuous neutral bath; we consider this clear evidence that a metabolic intoxication is the principal factor in thyroid crisis and that it may lead to a fatal issue even though the factor of hypercombustion be controlled. The present work indicates, pending proof to the contrary, that the most important site of disturbed intermediary metabolism is the liver. Whether a derangement of the metabolism of substances other than carbohydrates also occurs will be learned by the anticipated improvement in such other tests of liver function as that of hippuric acid excretion, as advocated by

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[2] Bartlett, W., Jr., *Tr. Section Surg., A.M.A.*, 1936.

Quick;[3] there is still controversy as to results obtained by its use in thyrotoxicosis.

Conclusions. Increased thyroid activity is accompanied by decrease in the amount of the blood amylase, and vice versa. There is a pronounced fall in the level of blood amylase within 8 hours following thyroidectomy in thyrotoxic patients; this does not occur after operations for non-toxic goiter. The return of blood amylase to normal values following thyroidectomy lags behind the improvement in clinical status and the restoration of basal metabolism to normal level. These events are considered to be evidence of impaired liver function in thyrotoxicosis.

9423

Action of Atropin and Eserin on Adrenalin Secretion Caused by KCl and CaCl.*

GERHARD KATZ AND GERTRUD KATZ. (Introduced by J. T. Halsey.)
From the Department of Pharmacology, Tulane University.

The introduction of $CaCl_2$ or KCl into the adrenal circulation is known to cause a secretion of adrenalin (Bacq and Rosenblueth,[1] Feldberg,[2] Katz and Katz[3]). In the present investigation we have endeavored to determine whether this adrenalin secretion in cats can be influenced by previous injections of atropin or eserin, assuming that atropin may decrease the amount of adrenalin secreted upon $CaCl_2$ or KCl injection, while eserin may enhance it. Such results would justify the working-assumption that the salts act on the adrenal medulla by means of an acetylcholin-like transmitter, as has been shown for the action of KCl on other organs such as salivary glands, tongue, sweat glands (Feldberg and Guimarais[4]), or for the transmission of splanchnic stimulation to the adrenals (Feldberg and coworkers[5, 6]).

Our method of recording the adrenalin output effected by the salts was the one developed by Feldberg and coworkers[7] and used in our previous investigations on $CaCl_2$ and KCl.[3] It consisted in comparing the effects on blood pressure and nictitating membrane produced by the salt when injected into a vein or into the central stump of the coeliac artery. The animal was eviscerated and with restricted circulation (aorta and vena cava tied at kidney level, the kidney vessels tied close to the kidneys). A small dose of the drug causes little or no effect upon intravenous injections, while the same amount injected into the central end of the coeliac artery reaches the adrenals in more or less concentrated form and may cause an adrenalin secretion. This latter adrenalin secretion manifests itself by a rise in blood pressure and a retraction of the denervated nictitating membrane after some latent period. These effects may be abolished by removal of the adrenals or enhanced by cocaine administration. They persist in decerebrated and pithed animals. Cats under dial anesthesia were used.

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[3] Quick, A. J., Arch. Int. Med., 1936, **57**, 544.

* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Bacq, Z. M., and Rosenblueth, A., *Am. J. Physiol.*, 1934, **108**, 46.

[2] See [4].

[3] Katz, G., and Katz, G., *J. Pharm. and Exp. Ther.*, 1937, **59**, 284.

[4] Feldberg, W., and Guimarais, J. A., *J. Physiol.*, 1936, **86**, 306.

[5] Feldberg, W., and Minz, B., *Pfluegers Arch.*, 1933, **233**, 657.

[6] Feldberg, W., Minz, B., and Tsudzimura, H., *J. Physiol.*, 1934, **81**, 286.

It was found that 0.2 mg. of atropin i.v. diminish the adrenalin secretion caused by Ca or K for a period of about 5 minutes (Fig. 1), while the effects of injected adrenalin remained unaltered. This short action of the atropin on the adrenals corresponds to the observation of Feldberg and Minz on adrenalin secretion caused by injected acetylcholin.[7] They found that an injection of acetylcholin discharged a decreased amount of adrenalin after intravenous injection of 2 mg. of atropin and that this effect lasted for about 2 minutes only.

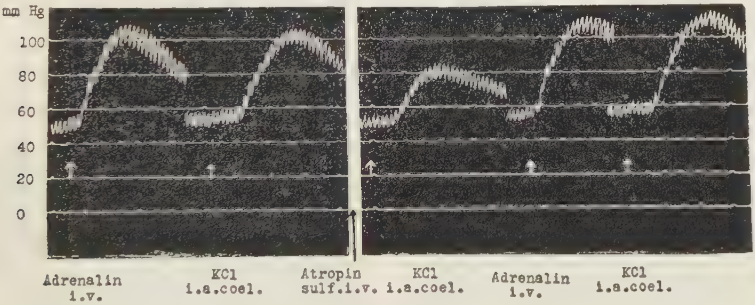

FIG. 1.

Eviscerated cat, Dial anesthesia, restricted circulation. Effects on blood pressure of 2.5γ adrenalin, intravenously, and 1.25 mg. KCl into central end of coeliac artery before and 2-10 min. after intravenous injection of 0.2 mg. atropin.

In our experiments, incidentally, we have often observed that atropin (1-2 mg. given intravenously) relaxes the denervated nictitating membrane. This observation is in contrast to Rosenblueth's report,[8] which stated that atropin has no action *per se* on the nictitating membrane. An explanation for this divergence may be suggested by the fact that we observed this effect only in membranes which were not completely relaxed at the time, while Rosenblueth may have studied the drug on relaxed membranes only.

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[7] Feldberg, W., and Minz, B., *Arch. Exp. Pharm.*, 1931, **163**, 66.

The effects of a previous injection of eserin (0.4-0.8 mg., intravenously) on the adrenalin secretion caused by injections of $CaCl_2$ or KCl have not been constant. In some experiments we have found no increase in response of blood pressure or nictitating membrane, while in others the reactions of both were enhanced, either in height or in length, although the effects of injected adrenalin were not modified (Figs. 2 and 3). However, in some experiments eserin unexpectedly enhanced the effects of injected adrenalin as well.†

The inconsistent findings in this part of our investigation, there-

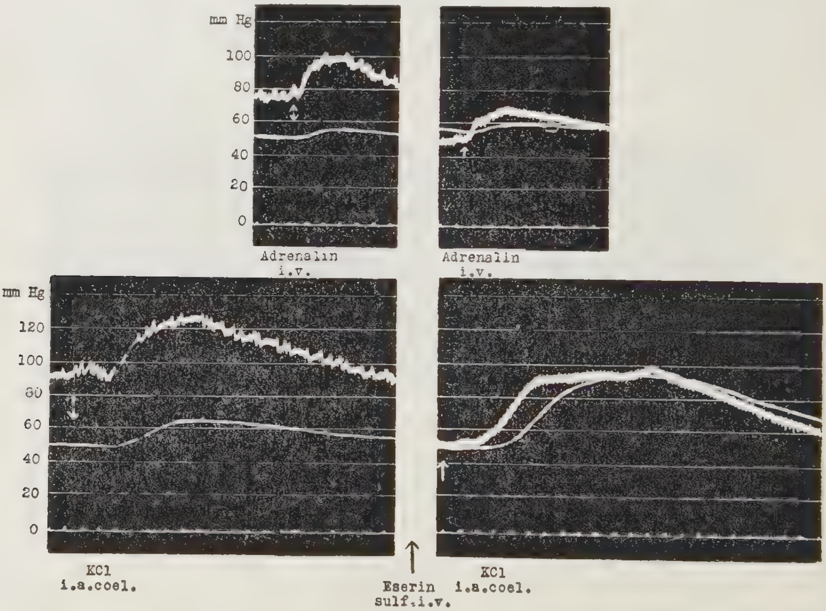

FIG. 2.

Upper curves: blood pressure; lower curves: acutely denervated nictitating membrane. Cat, Dial anesthesia. Preparation as in text. Effects of 5γ adrenalin, intravenously, and 5 mg. KCl into coeliac artery before and after intravenous injection of 0.5 mg. eserin sulf.

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[8] Rosenblueth, A., *Am. J. Physiol.*, 1932, **100**, 443.

† After this work was completed J. Secker (*J. Physiol.*, 1937, **89**, 296) reported that the action of adrenalin (like the effects of nervous stimulation) on the nictitating membranes of cats can be enhanced by previous administration of eserin; we observed this effect only occasionally.

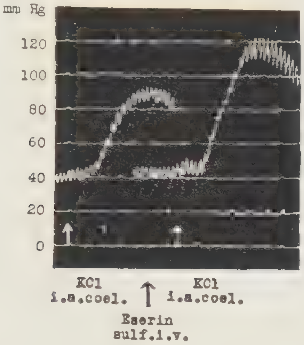

FIG. 3.

Cat. Preparation as in text. Effects on blood pressure of 1.25 mg. KCl injected into the central end of coeliac artery before and after intravenous injection of 0.4 mg. eserin sulfate.

fore, do not yet allow us to conclude that $CaCl_2$ or KCl discharge adrenalin by a cholinergic mechanism. Moreover, we do not believe that in studies of the autonomic nervous system, the effects of eserin and particularly those of atropin by themselves prove whether or not a mechanism is cholinergic. In our experiments, a cholinergic action of $CaCl_2$ or KCl on the adrenals can be definitely proved only by actual demonstration (by bioassay) of the liberation of acetylcholin in the glands following injection of the salts.

9424 P

Neurotoxic Action of Aluminum Salts.

HENRY W. SCHERP AND CHARLES F. CHURCH. (Introduced by C. F. Schmidt.)

From the Department of Pediatrics, School of Medicine, University of Pennsylvania, and Children's Hospital of Philadelphia.

In the course of experiments involving the fractionation of herpes virus preparations by aluminum salts it was observed that rabbits which had received an intracerebral injection of certain fractions developed a syndrome indicative of a lesion of the central nervous system. The possibility of herpes encephalitis was excluded by the dissimilarity in symptoms and in cellular pathology. Nevertheless, several features of this syndrome, which had a uniformly fatal termination, were suggestive of a neurotropic virus disease, and a further investigation was made of its etiology.

It was found that the characteristic syndrome may be induced in rabbits, mice and monkeys by a single intracerebral injection of a small amount of an aluminum salt. Siem[1] and Döllken[2] reported observing neurologic symptoms in rabbits, cats and dogs which had received subcutaneous injections of aluminum in the form of sodium aluminum tartrate or lactate. They found lesions in the central nervous system of these animals, particularly nerve cell degeneration in the lower cranial nerves. Seibert and Wells[3] reported lesions in the central nervous system of one of a series of rabbits to which large amounts of aluminum or its salts had been administered. Scant attention has been paid to the neurotoxic action of aluminum salts and no instance of intracerebral administration of these salts has come to our attention.

When the proper dose of aluminum salt is given intracerebrally, the animal recovers uneventfully from the injection. The subsequent course is afebrile, except for rare elevations of temperature which appear to be irrelevant. After a lapse of 7 to 10 days in the rabbit, the first characteristic symptom appears. This consists in the disinclination of the animal to resume the normal posture when the hind quarters are twisted through 90 degrees about the longitudinal axis. Normal rabbits resist this procedure vigorously. A distinct diminution in muscle tone accompanies this ataxia, which becomes progressively more severe until the animal is prostrate. Convulsions generally occur in the terminal stage, often accompanied by opisthotonus and spontaneous nystagmus. The animal becomes emaciated and dies 3 to 8 days after the onset of symptoms. The histopathology consists in widespread nerve cell changes of the type known as toxic degeneration, with slight inflammatory reaction. A total of 46 rabbits have received intracerebral injections of aluminum salts in this study. Of these, 5 died within 24 hours, all having received doses of one mg. or more of Al. Three animals remained free of symptoms, all having received 0.17 mg. or less. One rabbit showed symptoms and subsequently recovered. All the remaining animals developed the typical syndrome and either died or were sacrificed. The minimal effective dose for the rabbit was 0.17 mg. Eight mice received intracerebral injections of aluminum lactate, the minimal effective dose being 0.016 mg. of Al. Three monkeys, *Macaca mulatta* (rhesus), received 2.1 to 4.25 mg. of Al intracerebrally. All developed the syndrome in 21 to 24 days.

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[1] Siem, *Inaug. Diss.*, Dorpat, 1886.

[2] Döllken, *Arch. Exp. Path. u. Pharm.*, 1897, **40**, 98.

[3] Seibert, F. B., and Wells, H. G., *Arch. of Path.*, 1929, **8**, 230.

This syndrome has been produced by both the sulfate and the lactate of aluminum. The lactate has been used in most of the experiments because solutions of it may be buffered at pH 6.0 to 7.0 (phenol red indicator) without precipitation of aluminum hydroxide. Intracerebral injection of doses of sodium lactate solution (pH 6.3) chemically equivalent to the amount of aluminum used had no effect upon the experimental animals. Moreover, these same animals succumbed typically to subsequent intracerebral injection of aluminum lactate.

Cultures from the brains of typical cases on blood agar and in Difco brain-heart infusion broth remained sterile. The possibility was considered that the syndrome was caused by a latent virus infection which was activated in the presence of the aluminum. However, negative results were obtained in attempts to transmit the disease in series by intracerebral inoculation of suspensions of brain tissue, from rabbit to rabbit and from rabbit to monkey.

Aluminum determinations have been made on the central nervous system of rabbits sacrificed in the terminal stage. A modification of the aurin method of Eveleth and Myers[4] was used. The results show that 25 to 37% of the injected aluminum is retained in the central nervous system. Of this amount 50 to 70% was in the cerebral hemispheres, 17 to 29% in the pons, medulla and cerebellum, and 13 to 23% in the cord. Normal controls contained an insignificant amount of aluminum in the central nervous system.

9425

Blood Fats During the Dietary Production of Fatty Livers in Dogs.

EUNICE V. FLOCK AND JESSE L. BOLLMAN.

From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.

Studies of blood fats were made to determine if any change might be associated with the production of fatty livers in dogs which received a high fat diet. The lipids studied were the neutral fats of the plasma; which were determined by the volumetric method of Allen;[1] and cholesterol, by the Lieberman-Burchard method as used by Bloor.[2] Four dogs were kept on the high fat diet for 35 to 40 days. They were given the equivalent of 102 calories for each kilo of body weight daily, as 4 gm. of a standard mixed diet and 10 gm. of lard for each kilo of body weight. Three developed fatty liver while the fourth, which had received lipocaic[3] in addition to the fat diet, had a liver of normal fat content. Fasting blood samples taken at intervals fluctuated in the concentration of neutral fat, but always within normal range, and bore no relationship to the amount of fat in the liver (Table I).

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[4] Eveleth, D. F., and Myers, V. C., *J. Biol. Chem.*, 1936, **113**, 449.

[1] Allen, N. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 991.

[2] Bloor, W. R., *J. Biol. Chem.*, 1914, **19**, 1.

TABLE I.
Neutral Fats in Plasma* (mg. per 100 cc.) During Production of Fatty Livers by a High Fat Diet.

Days on diet	Dog			
	1	2	3	4†
1	258	211	230	76
7	118	106	218	114
13	146	185	277	217
20	161	130	304	210
26	135	114	209	217
28	264	244	342	394
32	321	260		
Liver fat, %	28.0	25.7	18.7	4.4

*Blood specimens taken 24 hours after feeding.
†Received lipocaic.

One day each week specimens of blood were taken at intervals, usually of 3, 6, 9, 12, 15, and 24 hours, following the regular fat meal. In spite of the high percentage of fat in the diet, which is known greatly to increase the emptying time of the stomach, definite lipemia occurred in 3 hours, particularly during the first few weeks that this diet was used. Later, perhaps because of intolerance to the diet, there may be no increase in neutral fat during this interval, or there may be even a decrease. Although in some instances the amount of postprandial fat in the blood was similar to that described by Bloor, Pelkan and Allen,[4] with a maximum in 6 hours, followed by a prompt return to the fasting level, often there were sporadic increases and decreases throughout the 24 hours. These are no doubt attributable to the irregular and prolonged absorption of fat which may occur with long-continued use of such a diet. A few of the typical curves are represented in Table II. Cholesterol in the plasma fluctuated within the normal range during these periods of 24 hours but apparently independently of either the blood fat or liver fat.

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[3] Dragstedt, *Am. J. Physiol.*, 1936, **117**, 171.

[4] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

TABLE II.
Neutral Fats in Plasma (mg. per 100 cc.) During Production of Fatty Livers.

Days on diet	Hours following fat meal						
	0	3	6	9	12	15	24
Dog 1—28% liver fat.							
1	258	397		446	360	276	237
13	146	261	263	124		315	104
26	135	380	313	347	254		202
Dog 2—25.7% liver fat.							
1	211	330		347	300	240	219
13	185	248	187	124		252	144
26	114	260	170	228	262		135
Dog 4—4.4% liver fat.*							
1	76	100	99	144	126	113	114
13	217	224	276	212		263	172
26	217	362	328	428	338		276

*Animal also received lipocaic.

Four dogs were given alcohol in addition to the fat diet since fatty livers can thus be produced more rapidly.[5] Fat curves of these dogs again showed values well within the normal range (Table III).

TABLE III.
Neutral Fats in Plasma (mg. per 100 cc.) of Dogs on a High Fat Diet Plus Alcohol.

Dog	Days on diet	Time after feeding, hours				Liver fat, %
		0	3	8	24	
5	14	344	263	291	272	20.7
6	8	300	203	308	202	11.1
7*	8	268	260	249	260	9.2
8*	14	327	392	417	257	6.5

*Received lipocaic.

Conclusions. The neutral fats of plasma are not elevated beyond the normal range during the production of fatty livers by a high fat diet or a fat diet plus alcohol. Fluctuations which do occur during the 24 hours following a meal are probably associated with varying rates of absorption.

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[5] Bollman, J. L., and Mann, F. C., *Arch. Path.*, 1935, **20**, 156.

9426

Histological Study of Thyroid Gland of Hypophysectomized Rats Exposed to Cold.*

OPAL WOLF AND ROY GREEP. (Introduced by F. L. Hisaw.)
From Goucher College and Harvard Biological Laboratories.

Smith[1] reported atrophy of the thyroid glands of frog tadpoles and rats[2] following the removal of the anterior lobe of the pituitary. Early death of the hypophysectomized animals was attributed chiefly to their inability to maintain normal body temperature because death did not occur when they were kept in specially heated cages. Smith concluded, "Thyroids of hypophysectomized tadpoles are undoubtedly entirely non-functional because they do not metamorphose. It seems probable that in hypophysectomized rats these glands are likewise not functional."

Various investigators have reported, however, that differences of temperature affect the activity of the thyroid gland. In recent extensive experiments Kuschinsky[3] found that the histological picture of the thyroid of rats at 38°-40°C. was that of an inactive gland and exposure to 4°C. resulted in great activity. Moreover, the thyrotropic factor in the pituitary glands of these rats decreased in the warm room and increased up to 25 to 30 days' exposure to the cold.

The changes brought about in the thyroid glands of animals subjected to cold might be explained by a nervous stimulation of the anterior lobe of the pituitary resulting in an increased output of the thyrotropic hormone and in addition the activity might be increased by a nervous stimulation of the thyroid.

Evidence for a nervous stimulation of the thyroid has been contradictory. Recently Bachromejew and Ter-Ossipowa[4] stimulated the peripheral endings of the superior laryngeal nerve of the cat and found activity in the thyroid gland as evidenced by cytological changes. In carefully controlled metabolism studies Haney[5] found that stimulation of the cervical sympathetic of rabbits resulted in an energy metabolism 22% above the normal at 5 days and concluded that the stimulation produced a secretory effect upon the thyroid gland. Nonidez,[6] however, found no secretory nerve endings in the thyroid of the dog but many terminations in walls of the blood vessels. He suggested that the blood flow through the gland is under the control of the nervous system and thus indirectly may influence the secretory phenomena and removal of the hormone produced by the gland.

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* This experiment was conducted at the Zoological Laboratory of the University of Wisconsin. We wish to thank Dr. Michael F. Guyer and Dr. Frederick L. Hisaw for extending to us the facilities of the laboratories.

[1] Smith, P. E., *Anat. Rec.*, 1916, **11**, 57.

[2] Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

[3] Kuschinsky, G., *Arch. f. Exp. Path. u. Pharmakol.*, 1935, **179**, 726.

[4] Bachromejew, I. R., and Ter-Ossipowa, N. A., *Endokrinologie*, 1935, **15**, 404.

[5] Haney, H. F., *Am. J. Physiol.*, 1932, **102**, 249.

The present investigation was undertaken to study the histological picture of the thyroid glands of young albino male rats which were hypophysectomized and subsequently placed in a cold room at 2°-4.5°C.

Seven young male rats 22 days of age were placed in a lighted cold room at a temperature of 2° to 4.5°C. for a week, hypophysectomized, kept in a warm room for 2 days and returned again to the cold room. Thirty-one males of the same age which had been kept in the warm animal room were hypophysectomized and on the third day 13 were placed in the cold room and 18 were left in the warm room. Each cage contained one or 2 normal animals. These experiments were carried on during the summer months so that the temperature varied in the animal room, reaching 29°C. during the middle of the day. Some of the controls were kept at room temperature in a small room which was lighted continuously. However, the animals in these lighted rooms, both cold and warm, were given ample cotton waste so that they could avoid the light. Immediately after hypophysectomy the animals were given access to glucose and water for several hours and then given the regular food consisting of ground grain mixed with a high percentage of brewer's yeast. In addition, a dish of glucose was kept in the cages. A large amount of brewer's yeast was given to stimulate the appetite of the animals and glucose was placed in the cage so that there would be a carbohydrate available which could be quickly assimilated.

At the conclusion of the experiment the thyroid and adrenal glands were fixed in Bouin's, dehydrated by the dioxan method,[7] stained in Delafield's hematoxylin and eosin and cut in serial sections at 7 micra. The sella of each animal was examined under a binocular for evidence of incomplete hypophysectomy and serial sections were made of those which survived the longest.

Two of the hypophysectomized rats (checked by serial sections through the sellae) were killed after surviving 34 days in the cold room. With the exception of 3 animals killed on the 10th day, 10 animals died after 2-10 days' exposure. Five survived 12 to 16 days and 2 were killed on the 34th day of exposure. The animals which survived longest in the cold had been subjected to the cold one week before hypophysectomy.

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[6] Nonidez, Jose F., *Am. J. Anat.*, 1935, **57**, 135.

[7] Guyer, M. F., *Animal Micrology*, Univ. of Chicago Press, Chicago, Ill., fourth edition.

Those animals which survived the longest were able to keep the body temperature at levels slightly below normal rats in the cold for 16 days, followed, however, by a gradual fall until just before death, when a drop of 1.6°-2.8° occurred.

The rectal temperatures of the animals were as follows:

Normal animals at room temperature	37.75°-38.3 °C.
” ” in cold (at 2° to 4.5°C.)	36.4 °-37.2 °C.
Hypophysectomized animals 16 days in cold	35 °-36.15°C.
” ” at room temperature	35.3 °-36.15°C.
” ” 20 days in cold	34.65°-35.35°C.
” ” 29 ” ” ”	34 °-35.45°C.
” ” 34 ” ” ”	32.65°-33.35°C.
” ” 33 ” at room temperature	36.55°

The 2 animals which survived 34 days in the cold (age 64 days and weighing 64 and 59 gm.) felt cold to the touch, showed asthenia and had not eaten for some time.

The thyroid glands of the normal controls in the cold showed a high columnar epithelium, loss of colloid in the small vesicles and in the larger vesicles many absorption vacuoles were present in the periphery of the colloid. Normal control animals in the warm room did not have as high an epithelium or as many absorption vacuoles as the animals in the cold.

Hypophysectomized rats in the warm room had atrophic glands with very low cuboidal to squamous epithelium. However, in the peripheral vesicles a few showed a high cuboidal epithelium and absorption vacuoles. The hypophysectomized animals in the cold room (killed on the 34th day) had glands which were atrophic in the center with small vesicles showing a low epithelium and no absorption vacuoles but the peripheral vesicles had high cuboidal to columnar epithelium. A considerable loss of colloid was seen in some vesicles and in others many absorption vacuoles were present in the periphery of the colloid. The adrenals of these animals also showed some atrophy. The cytoplasm of the cells of the *zona fasciculata* in places had very large vacuoles and there was a wide area with pycnotic nuclei in the *zona reticularis*.

It is of interest to note that surviving hypophysectomized rats were able to maintain body temperature for 16 days in the cold at approximately the same level as similar rats in a warm room. Normal rats are imperfectly homoiothermic. In the cold room rectal temperature dropped to the level of the rats hypophysectomized and placed either in the cold or heat for 16 days. That cold stimulates the metabolism of rats in the absence of the thyroid gland has been shown by Ring's[8] studies. However, Grollman and Firor[9] found that in a warm room the fall in body temperature of white rats with chronic adrenal insufficiency was due to the failure of the anterior lobe of the pituitary to secrete sufficient thyrotropic hormone. By injections of either a thyrotropic hormone, or the feeding of thyroxin they maintained body temperature at approximately normal levels. However, it has been shown by Hartman, *et al.*,[10] and Cannon, *et al.*,[11] that epinephrine is secreted when animals are subjected to cold and the lack of this hormone may be one of the contributing factors in causing the early death of recently adrenalectomized animals which were exposed to the cold.†

In the present experiment the ability of surviving hypophysectomized animals in the cold to maintain body temperature for 16 days at nearly the level of the normal rats at the same temperature indicates a secretion of hormone from the thyroid gland. However, the histological picture of the thyroid gland of the animals 34 days in the cold did not present a picture comparable to that of normal animals at the same temperature. The removal of the anterior lobe of the pituitary had resulted in an atrophy in the central region of the gland. That some hormone was still being released was probable because some peripheral follicles showed considerable loss of colloid, others showed the typical high epithelium with absorption pseudopods or absorption vacuoles in the periphery of the colloid as described by Sevringhaus.[12] This picture is not characteristic of the thyroid gland of hypophysectomized animals. It is evident, however, from this experiment that cold in the absence of the anterior lobe of the pituitary is not an adequate stimulus to release sufficient hormone from the thyroid gland to maintain a temperature characteristic of a normal rat in the cold.

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[8] Ring, G. C., *Abst. Am. J. Physiol.*, 1936, **116**, 129.

[9] Grollman, A., and Firor, W. M., *Am. J. Physiol.*, 1935, **112**, 310.

[10] Hartman, F. A., McCordock, H. A., and Loder, M. M., *Am. J. Physiol.*, 1923, **64**, 1.

[11] Cannon, W. B., and Querido, A., *Proc. Nat. Acad. Sciences*, 1924, **10**, 245.

† During this experiment 2 adult male rats doubly adrenalectomized 1½ days (19 days having elapsed between the removal of the right and left gland, one animal having gained 20, the other 15 gm. by the day of the second (left) adrenalectomy) were placed in the cold room. At the end of 7 hours one animal was dead and the temperature of the other dropped from 36.5° to 26.25°C. This animal was returned to room temperature and at the end of 12 hours had a temperature of 25.5°C. and died 8 hours later. Unpublished data of Robert Kroc.

[12] Sevringhaus, A. E., *Z. f. Zellforsch. u. Mik. Anat.*, 1933, **19**, 653.

The fall in temperature, asthenia and failure to grow indicates undoubtedly a multi-glandular disfunction due to hypophyseal insufficency.

Summary. 1. Normal young albino rats had a temperature of 36.4°-37.2°C. in the cold, in a warm room 37.7°-38.3°C. 2. Surviving hypophysectomized rats maintained their body temperature for 16 days in a cold room (2°-4.5°C.) at 35°-36.15°C. From the 20th to 34th days the temperature fell approximately 1.3°-2.7°C. 3. The thyroid glands of normal animals in the cold showed activity as previously described by other investigators. Those in the warm room had a lower epithelium and fewer absorption vacuoles. 4. The glands of hypophysectomized rats 38 days in the heat showed atrophy. Similar animals placed in the cold room 34 days showed signs of atrophy in the central part of the gland but absorption of colloid was indicated in the peripheral vesicles by the high epithelium, loss of colloid in the smaller vesicles, and many absorption vacuoles in the peripheral colloid of the larger vesicles. 5. The drop in body temperature of the hypophysectomized rats after 16 days in the cold as well as the histological picture of the thyroid gland indicates that cold in the absence of the anterior lobe of the pituitary is not an adequate stimulus to maintain either body temperature or a histological picture of the thyroid gland comparable to that of a normal rat.

9427

Effect of Calcium on the Digitalized Heart.*

L. H. NAHUM AND H. E. HOFF. (Introduced by J. F. Fulton.)
From the Laboratory of Physiology, Yale University School of Medicine, New Haven.

The action of calcium upon the mammalian heart has certain similarities to that of digitalis. Slowing of the heart rate, A-V block of varying degree, and ectopic beats have been observed following administration of either of these substances in adequate amounts, and have given rise to a generally accepted opinion that they both operate, at least in part, through a common mechanism. This has prompted attempts to detect additive or synergistic activities when both were given simultaneously. The studies of Billigheimer[1] and of Lieberman[2] support such a conclusion, and Bower and Mengle[3] explain the sudden death of patients given calcium chloride intravenously while under the influence of digitalis, on the basis of such additive effects.

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* This work was aided by a grant from the Research Funds of the Yale University School of Medicine.

The experiments upon which these conclusions have been based, however, involve doses of digitalis considerably greater than are usually employed in man, and in some experiments such large quantities were used that it may be questioned whether the entire phenomenon was not in fact due to the administration of lethal doses of digitalis. The death of patients reported by Bower and Mengle can further not be correctly assigned to an additive action of digitalis and calcium as long as the influence of calcium upon the damaged heart requiring digitalis is unknown. It seemed desirable, therefore, to determine the effect of carefully standardized doses of calcium chloride upon the heart of normal, unanesthetized animals, that had received digitalis in therapeutic and sub-lethal amounts.

Twenty-five unanesthetized rabbits weighing from 1.4 to 2.1 kg. were employed. In 12 the amount of calcium required to arrest the heart was first determined in the normal animal, and again several days later after they had received from ½ to ¾ of the calculated lethal dose of digitalis. Two of the remaining 13 animals served as controls of the effect of the calcium alone for 11 rabbits from the same group which were digitalized previous to calcium administration.

Digitalis was given in the form of digifoline, which contained one cat unit in 2.0 cc. Eighteen rabbits were given ½ to ¾ cat units per kg. subcutaneously 18 hours before calcium was administered. Five other rabbits received one-half cat unit per kg. in a similar manner, and were given an additional intravenous injection of ¼ cat unit per kg. one-half hour before the calcium was given.

A 10% solution of calcium chloride was injected intravenously at a rate of 2.0 cc. per minute.

Electrocardiograms were taken from Lead II after injection of each successive cubic centimeter of calcium chloride, and at brief intervals after cessation of injections.

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[1] Billigheimer, E., *Klin. Wchnschr.*, 1929, **8**, 724.

[2] Lieberman, A. L., *J. Pharm. and Exp. Therap.*, 1933, **47**, 183.

[3] Bower, J. O., and Mengle, H. A. R., *J. Am. Med. Assn.*, 1936, **106**, 1151.

The effect of calcium upon the 14 normal unanesthetized rabbits were in every respect identical with those previously reported from these laboratories.[4] In brief, they consisted of (1) typical changes in the ventricular complex of the electrocardiogram; (2) slowing in rate of the heart and delay in A-V conduction, becoming more pronounced with increasing quantities of calcium, (3) auricular fibrillation and ectopic ventricular beats, and finally, with continued injection of calcium, cardiac arrest. The quantities of calcium required to arrest the heart varied from 1 to 5 cc., and did not seem to depend upon variations in size of the animal, within the limits shown in Table I.

TABLE I.

Exp. No.	Wt., kg.	Amount of 10% calcium chloride in cc. required to arrest heart		Cat units of digifoline given	Cat units per kg.
		A. Normal	B. Digitalized		
82	1.8	3.0	3.0	1.0	1/2+
83	2.1	3.0	3.0	1.0	1/2—
84	1.5	3.0	3.0	1.0	2/3
85	1.4	2.0	2.0	0.75	1/2
86	1.8	3.0	3.0	1.0	1/2+
87	1.8	4.0	3.0	1.0	1/2+
88	1.6	4.0	4.0	1.2	3/4
89	1.2	1.0	2.0	0.9	3/4
90	1.5	4.0	4.0	1.15	3/4
91	1.6	5.0	4.0	1.2	3/4
92	1.8	2.0	3.0	1.35	3/4
93	1.7	1.5	3.0	1.3	3/4

The effects of injections of calcium upon digitalized hearts were not to be distinguished from those occurring in the normal heart. Similar arrhythmias were produced by the same amounts of calcium in both normal and digitalized hearts, and it required the same total quantity of calcium to arrest the digitalized heart as the normal heart. The total duration of the calcium effect varied in both from 3 to 10 minutes (Fig. 1 Table I.)

Three of the 5 animals which received an additional intravenous injection of digitalis a half hour before the calcium died about 4 hours later.

These experiments indicate that in unanesthetized rabbits, the amount of calcium required to arrest the thoroughly digitalized heart is no less than that necessary in a non-digitalized heart. Further, the arrhythmias produced by calcium in the digitalized

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[4] Hoff, H. E., and Nahum, L. H., *J. Pharm. and Exp. Therap.*, 1937, in press.

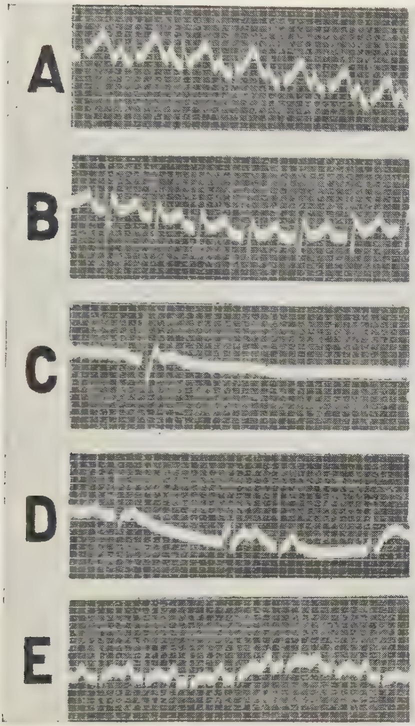

Fig. 1.

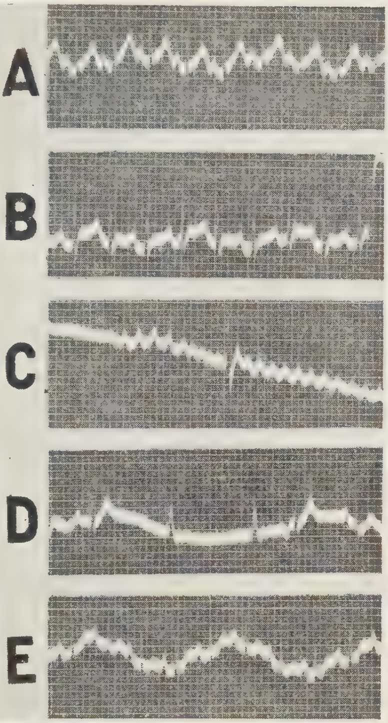

Fig. 2.

Fig. 1.

Rabbit 82. Before digitalization. 3 cc. 10% $CaCl_2$ intravenously. 2 cc. per min. Lead II. A Control. B. After injection of 2 cc $CaCl_2$. C. Immediately after injection of 3 cc. D and E. Records at 30-second intervals, showing recovery.

Fig. 2.

Rabbit 82, 7 days after Fig. 1. Given ½ cat units digifoline per kg. subcutaneously 18 hours previously. A. Control. B. After 2 cc. $CaCl_2$. C. After 3 cc. $CaCl_2$, showing ventricular arrest with auricular flutter. D and E. Records at 30-second intervals showing recovery.

heart are the same as are produced in the normal heart by the same amount of calcium. Finally the digitalized heart does not exhibit any evidence of an increased tendency to develop ventricular rhythms.

The amounts of digitalis administered in these experiments were greater than the usual doses employed in clinical medicine, and 3 animals receiving the greatest amounts died several hours later. Failure to obtain so-called additive effects in these animals cannot, therefore, be attributed to inadequate amounts of digitalis. Similarly calcium was administered in amounts which actually produced arrest in control experiments on the same animals several days before they were digitalized, so that obviously adequate quantities of calcium were given.

The absence of additive phenomena in these experiments does not permit the assumption that these substances may be administered with impunity. The toxic effects of digitalis are well known, and those of calcium are also being recognized. In digitalis administration the presence of a damaged heart in which sudden circulatory accidents may occur, is usually a complicating factor. Likewise, calcium alone produces a wide range of cardiac arrhythmias, some of which might well prove fatal in the presence of preëxisting cardiac damage. It has been observed that atropinized hearts occasionally develop ventricular fibrillation with calcium, and it is possible that hearts which because of disease show a tendency to ectopic ventricular rhythms might be set into ventricular fibrillation by amounts of calcium which produce only transient disturbances in a normal heart. This may be the mechanism of sudden death reported by Wolffe and Bellet which occurred during administration of calcium to a patient suffering from paroxysmal tachycardia.[5]

Conclusion. In the normal unanesthetized rabbit heart the effects of calcium and digitalis are not additive.

9428 P

Total Coproporphyrin I Excretion in Pernicious Anemia.

Konrad Dobriner and W. Halsey Barker. (Introduced by C. P. Rhoads.)

From the Hospital of the Rockefeller Institute for Medical Research.

The mechanism of the disturbed metabolism of the respiratory pigments in pernicious anemia has been a matter of controversy.[1-4] Theoretically, qualitative and quantitative disturbance may occur at any stage in the construction and destruction of the hemoglobin molecule, but it is not clear whether the increased bile-pigment production and excretion is consequent to a quantitatively increased destruction of hemoglobin similar to that of hemolytic jaundice, or whether it is due to a partial or total pathologic metabolism of the precursors of hemoglobin.

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[5] Wolffe, J. B., and Bellet, S., *Ann. Int. Med.*, 1930-31, **4**, 794.

[1] Addis, T., *Arch. Int. Med.*, 1915, **15**, 413.

[2] Eppinger, H., *Die hepatolienalen Erkrankungen*, Berlin, 1920.

[3] Whipple, G. H., *Arch. Int. Med.*, 1922, **29**, 711.

[4] Rous, P., *Phys. Rev.*, 1923, **3**, 75.

Porphyrin and urobilin excretion in a case of pernicious anemia have been investigated quantitatively. It has been reported that an increased coproporphyrin I excretion in both the urine and feces is present during relapse, and that lower values are present during remissions.[5, 6, 7] However, because of the lack of adequate methods the reported data are inconclusive. Watson[8] reported adequate data on feces, but omitted determinations of the porphyrin in the urine.

Case History. A 61-year-old white male entered with anemia of 4 years' duration. Inadequate treatment with liver extract had been employed and the disease had progressed. Physical examination showed a subicteric tint of the skin, emaciation, and some atrophy of the papillae of the tongue. Examination of the blood on admission showed 900,000 R.B.C., hemoglobin 23%, mean corpuscular volume 1.13, color index 1.21, icterus index, 7, and W.B.C. 3,250. The blood smear and the histologic picture of the bone marrow were characteristic of pernicious anemia. Gastric analysis failed to reveal free hydrochloric acid, even after histamine. Details of treatment, and hematologic response are briefly summarized in Chart I. The patient received an injection of 5.0 cc. of liver extract (Lederle & Co. concentrate) and 10.0 cc. of liver extract (Eli Lilly & Co.) intramuscularly on the 16th and 17th days of observation respectively.

The methods used for porphyrin separation and quantitative measurement were similar to those previously described.[9, 12] The urobilin was determined as urobilinogen by the Watson modification of the Terwen method.[10] The coproporphyrin excreted was identified as coproporphyrin I by melting-point determination.

Quantitative coproporphyrin and urobilin determinations were made during the periods of relapse, treatment, and remission (Text Fig. 1). The total coproporphyrin output during the 15-day control period in relapse averaged 632 gamma per diem and the urobilinogen averaged 442 mg. Shortly after therapy was instituted the reticulocytes rose rapidly to 60%; during this period there was little change in the total coproporphyrin and urobilinogen output (611 micrograms coproporphyrin and 533 mg. urobilinogen daily average). The amount of coproporphyrin in the urine decreased from 158 to 100 micrograms. After the reticulocyte response was completed and 7 days after the onset of therapy the total amount of coproporphyrin

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[5] Duesberg, R., *Arch. exp. Path. u. Pharm.*, 1931, **162**, 268.

[6] Watson, C. J., *J. Clin. Inv.*, 1935, **14**, 116.

[7] Brugsch, J., *Th. Z. ges. exp. Med.*, 1935, **95**, 490.

[8] Watson, C. J., *J. Clin. Inv.*, 1937, **17**, 389.

[10] Watson, C. J., *Arch. Int. Med.*, 1931, **47**, 698.

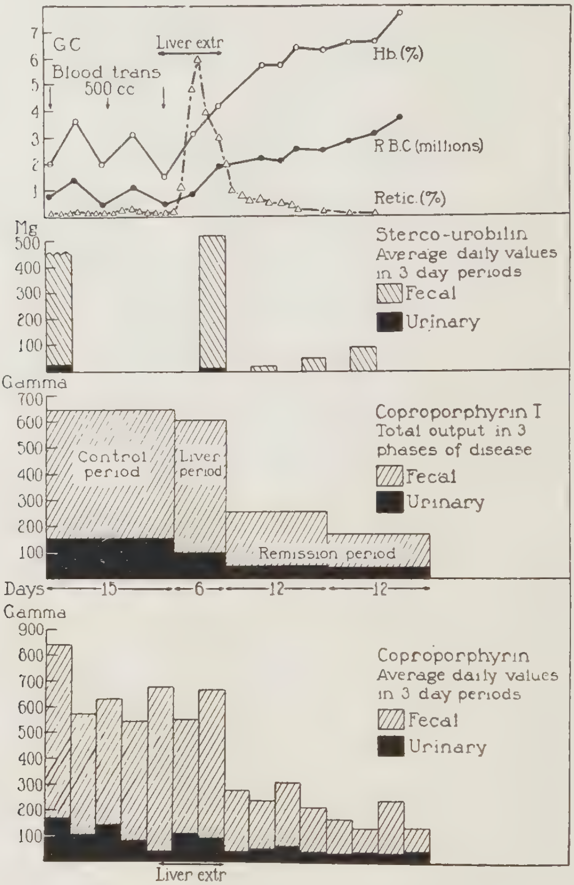

FIG. 1.

excreted decreased rapidly to 261 and 172 micrograms daily average and for the urobilinogen to 40 mg. and 93 mg. respectively in the next two 12-day periods. Increased protoporphyrin was excreted in the feces.

Previously published work has demonstrated the close relationship between coproporphyrin I excretion and construction of the respiratory pigment, and has shown evidence of a proportional as well as a disproportional construction of Type I and Type III porphyrins in various pathological conditions.[9, 11, 12, 13] Whereas a definite dis-

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[9] Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1, and in press.

[11] Dobriner K., Localio, S. A., Strain, W. H., *J. Biol. Chem.*, 1936, **114**, XXVI.

[12] Dobriner, K., Strain, W. H., Localio, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 752, 755.

[13] Dobriner, K., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 757.

turbance of pigment construction in pernicious anemia is indicated by this study, it is not possible to state whether the observed increased coproporphyrin I excretion is a simple increase of a normal process or is an abnormal or disproportional type of construction such as is present in porphyria.[11]

The levels of coproporphyrin I and urobilin excretions in pernicious anemia are similar to those reported for hemolytic jaundice.[12] Moreover, the changes in the rate of output after specific therapy in the two conditions are alike. These facts suggest that the increased coproporphyrin I excretion in pernicious anemia during relapse reflects a normally increased production proportional to an increased Type III porphyrin formation rather than a pathological production resulting in a disturbed ratio between the formation of Type I and Type III compounds.

9429

Intravenous Injection of Aminoacids in Regeneration of Serum Protein Following Severe Experimental Hemorrhage.

ROBERT ELMAN. (Introduced by E. A. Graham.)

From Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis.

Nine pairs of experiments were performed, dogs of the same size, sex and condition being used as far as possible for each experiment. Under local anesthesia 3.5% of the body weight was bled from the femoral artery and immediately the same volume of Ringer's solution replaced into the femoral vein. One half hour later the first specimen of blood was taken from the jugular vein. Each dog was then given intravenously over the course of 3 hours a volume of fluid equal to 3.5% of the body weight. To one, 10% glucose in Ringer's was given; to the other 5% glucose plus 5% aminoacid mixture in Ringer's. Six hours after the beginning of the injection a second sample of jugular blood was taken; a third specimen was obtained 24 hours later when the experiment was finished. Additional samples of blood were taken in all animals to determine the level of its aminoacid content. No symptoms were produced by the injections and the dogs remained in good condition throughout the period of observation. Though allowed water, all food was withheld, not only during the experiments but for a period of 48 to 72 hours previously. The slow rate of injection of the aminoacids raised the aminoacid nitrogen of the blood to not over 11 mg./100 cc.; one half hour after the end of the injection it returned to the normal level, about 6 mg. Only about 10% of the injected aminoacids appeared in the urine. The Folin colorimetric method was used for determining aminoacid nitrogen.

The blood samples were measured for cell volume and total protein. Hematocrits were done by centrifuging whole blood rendered incoagulable with prontamine fast pink Bl—one mg. per cc.; a second sample was centrifuged after adding 5 cc. to one cc. of 1.6% sodium oxalate. The values obtained checked usually within 1%, never greater than 3%. Serum protein was determined by the macro-Kjeldahl-titration method and was done in duplicate, checks within 2% being obtained. The value for non-protein nitrogen was always the same, but was first subtracted from the total nitrogen before calculating the protein value.

The mixture of aminoacids used was obtained through the courtesy of Mead Johnson and Company and with the continued coöperation of Drs. Cox and Kemmerer of their staff. It was prepared by hydrolyzing purified casein; tryptophane was added, 2% by weight, and the mixture dissolved in Ringer's solution and filtered before use. It formed a clear, light amber solution of a pH of 5.8, was not anaphylactogenic for guinea pigs and showed no precipitate when treated with the tungstic acid reagent.

CHART I.

Actual Serum Protein Changes* (gm. per 100 cc. serum or plasma).

	Glucose Injections			Aminoacid Injections		
Exp.	Start	6 hr.	24 hr.	Start	6 hr.	24 hr.
1	5.40	—.40	—.10	4.0	+.60	xx
2	5.30	—.10	+.10	4.50	+1.00	+.30
3	4.90	+.20	—.10	4.90	+.50	+.90
4	5.20	—.30	+.30	5.40	+.10	+.30
5	4.19	—.20	+.06	4.40	+.26	+.57
6	4.86	—.20	+.34	4.46	+.31	+.61
7	4.59	+.35	+.31	4.37	+.26	+1.40
8	4.68	+.30	+.35	4.72	+.22	+.79
9	4.24	—.44	+.13	5.11	+.18	+.31
Av.		—.09	+.15		+.38	+.64
%		—2	+3		+9	+14

*Changes are indicated by + or — and are obtained at both 6 and 24 hrs. by subtraction from the original ("start") value.

The findings are recorded in Chart I. It will be seen that whereas the animals treated with glucose alone showed no regeneration after 6 hours and little even after 24 hours, the dogs treated with aminoacids and glucose exhibited a definite rise in 6 hours and a marked one in 24 hours. Lest this increased value be due to a concentration of the blood it was necessary to consider any changes in the total volume of circulating plasma. Direct measurements of the plasma volume with the vital red technic did not give accurate results, owing to difficulty in colorimetric readings although no essential differences between the glucose and aminoacid animals were observed. To get an approximate figure for the total plasma proteins the hematocrit was used, assuming that the total cell volume did not change during the period of observation or at least that similar changes occurred in the glucose and the aminoacid animals. That no essential change occurred in the size or pigment content of the red cells was indicated by a close correlation between the erythrocyte count, hemoglobin value (Sahli) and hematocrit, all of which showed a similar drop indicating an increase in the relative plasma volume, *i. e.*, dilution of the whole blood. The results obtained by calculating the relative total plasma protein changes are indicated in Chart II; it will be seen that here too the increase of plasma protein is far greater in the aminoacid dogs than in those receiving glucose alone. The method of calculation was as follows: for the initial value, the plasma volume (hematocrit reading) was multiplied by the serum protein; this was taken as the amount of protein per 100 cc. of blood at the start. Subsequent plasma volumes per 100 cc. of this original blood were obtained by multiplying the actual plasma volume observed in the hematocrit by a factor which corrected for the observed change in relative cell volume (hematocrit reading). Thus, if the relative cell volume changed from 35 to 30, the plasma volume (per 100 cc. of the original blood "at the start") changed from 65 to 70 × 35/30. The plasma volume thus obtained was multiplied by

CHART II.
Relative Total Plasma Protein Changes* (% of initial value).

	Glucose Injections		Aminoacid Injections	
Exp.	6 hr.	24 hr.	6 hr.	24 hr.
1	—6	+8	+48	xx
2	+18	+25	+47	+42
3	+27	+31	+66	+78
4	+18	+21	+48	+39
5	+30	+40	+70	+87
6	+28	+45	+57	+54
7	+31	+33	+22	+51
8	+25	+30	+30	+61
9	+ 9	+32	+24	+40
Av.	+20	+29	+46	+57

*Changes are calculated at both 6 and 24 hours from the original ("start") value which is taken as 100%.

its serum protein concentration and the change expressed as a percentage of the initial value (Chart II).

Summary. Healthy starving dogs subjected to a single severe hemorrhage revealed a much more striking regeneration of serum protein in 6 and 24 hours when treated by intravenous injection of a complete mixture of aminoacids and glucose than of glucose alone. The findings suggest that the aminoacids introduced in this way are utilized in the regeneration of serum protein. Clinical observations in patients unable to take protein by mouth have given similar results; nitrogen balance observations on them as well as on animals are now being carried out.

The author is indebted to Dr. H. L. White for many helpful suggestions during the course of this study.

SECRETARY'S REPORT

April 1, 1936 to April 1, 1937

During the past academic year we have progressively adopted more provisions of the new constitution. Beginning next September the new constitution will prevail.

The following is a concise statement of basic facts.

OFFICERS elected April 21, 1937

President: H. S. Gasser.
Vice-President: J. T. Wearn.
Secretary-Treasurer: A. J. Goldforb.
Council: C. D. Leake (2 years).
W. J. Meek (2 years).
H. W. Smith (4 years).
E. A. Doisy (4 years).
Past President (by constitution): P. E. Smith.
Chairmen of Sections (see Sections).

NATIONAL COMMITTEES

Nominating Committee (by constitution): The Council.

Editorial Committee (appointed by Council): D. W. Bronk, G. W. Corner, C. H. Danforth, A. B. Hastings, S. B. Hooker, A. C. Ivy, H. B. Lewis, A. B. Luckhardt, W. S. McCann, W. DeB. MacNider, A. M. Pappenheimer, O. H. Plant, T. M. Rivers, H. B. Williams, A. J. Goldforb.

Membership Committee (appointed by Council): J. Auer, C. F. Craig, J. A. Dye, H. Goldblatt, I. Greenwald, P. J. Hanzlik, A. C. Ivy, W. P. Larson, E. D. Plass.

Finance Committee: W. J. Gies, W. H. Park, A. J. Goldforb.

PAST OFFICERS

Date	*President*	*Vice-President*	*Secretary*	*Treasurer*
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	" "	" "
1905-06	E. B. Wilson	E. K. Dunham	" "	" "
1906-07	S. Flexner	E. K. Dunham	" "	" "
1907-08	S. Flexner	T. H. Morgan	" "	" "
1908-09	F. S. Lee	T. H. Morgan	" "	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	" "
1910-11	T. H. Morgan	W. J. Gies	" "	" "
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	" "
1912-13	J. Ewing	P. A. Levene	" "	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	" "
1914-15	G. Lusk	W. J. Gies	" "	J. R. Murlin

Date	*President*	*Vice-President*	*Secretary-Treasurer*
1915-16	G. Lusk	G. N. Calkins	H. C. Jackson
1916-17	J. Loeb	W. J. Gies	" "
1917-18	W. J. Gies	J. Auer	" "
1918-19	W. J. Gies	J. Auer	" "
1919-20	G. N. Calkins	G. B. Wallace	" "
1920-21	G. N. Calkins	G. B. Wallace	" "
1921-22	G. B. Wallace	J. W. Jobling	" "
1922-23	G. B. Wallace	J. W. Jobling	" "
1923-24	H. C. Jackson	J. W. Jobling	V. C. Myers
1924-25	H. C. Jackson	J. W. Jobling	A. J. Goldforb
1925-26	J. W. Jobling	S. R. Benedict	" "
1926-27	J. W. Jobling	S. R. Benedict	" "
1927-28	S. R. Benedict	P. Rous	" "
1928-29	S. R. Benedict	P. Rous	" "
1929-30	P. Rous	D. Marine	" "
1930-31	P. Rous	D. J. Edwards	" "
1931-32	D. J. Edwards	A. R. Dochez	" "
1932-33	A. R. Dochez	E. L. Opie	" "
1933-34	A. R. Dochez	E. L. Opie	" "
1934-35	E. L. Opie	P. E. Smith	" "
1935-36	E. L. Opie	P. E Smith	" "
1936-37	P. E. Smith	E. F DuBois	" "

SECTIONAL MEETINGS AND MEMBERSHIP

Cleveland, Ohio

Chairman: A. R. Moritz. Secretary: E. Muntwyler. Members: 41.
Meetings: Western Reserve University, November 13, 1936.
Western Reserve University, March 12, 1937.
Western Reserve University, May 14, 1937.

District of Columbia

Chairman: T. Koppanyi. Secretary: M. E. Maver. Members: 28.
Meetings: George Washington University, January 28, 1937.
George Washington University, May 27, 1937.

Illinois

Chairman: L. R. Dragstedt. Secretary: L. A. Crandall, Jr. Members: 115.
Meetings: Chicago University Medical School, November 25, 1936.
University of Illinois Medical School, February 9, 1937.
Northwestern University Medical School, April 13, 1937.
Chicago University Medical School, June 1, 1937.

Iowa

Chairman: C. P. Berg. Secretary: W. R. Ingram. Members: 36.
Meetings: State University of Iowa, November 24, 1936.
State University of Iowa, February 25, 1937.
State University of Iowa, May 13, 1937.

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 47.
Meetings: University of Minnesota, February 17, 1937.

Missouri

Chairman: G. H. Bishop. Secretary: A. B. Hertzman. Members: 52.
Meetings: Washington University Medical School, October 14, 1936.
St. Louis University Medical School, November 11, 1936.
Washington University Medical School, February 10, 1937.
St. Louis University Medical School, March 10, 1937.
Washington University Medical School, April 14, 1937.
St. Louis University Medical School, May 12, 1937.

New York

Chairman: H. S. Gasser. Secretary: I. Greenwald. Members: 732.
Meetings: New York Academy of Medicine, October 21, 1936.
New York Academy of Medicine, November 18, 1936.
New York Academy of Medicine, December 16, 1936.
New York Academy of Medicine, January 20, 1937.
New York Academy of Medicine, March 17, 1937.
New York Academy of Medicine, April 21, 1937.
New York Academy of Medicine, May 19, 1937.

Pacific Coast

Chairman: C. D. Leake. Secretary: J. Field II. Members: 95.
Meetings: Stanford University, October 10, 1936.
University of California Medical School, December 9, 1936.
Stanford University Medical School, February 3, 1937.
Mount Zion Hospital, March 10, 1937.
University of California, May 8, 1937.

Peiping (China)

Chairman: S. H. Liu. Secretary: C. H. Hu. Members: 37.
Meetings: Peiping Union Medical College, October 28, 1936.
Peiping Union Medical College, February 24, 1937.

Southern

Chairman: A. Ochsner. Secretary: R. Ashman. Members: 36.
Meetings: Tulane University, December 4, 1936.
Tulane University, March 5, 1937.
Tulane University, May 21, 1937.

Southern California

Chairman: G. H. Ball. Secretary: C. H. Thienes. Members: 36.
Meetings: University of Southern California, October 21, 1936.
University of Southern California, December 29, 1936.
Scripps Institution, February 27, 1937.
University of California at Los Angeles, May 3, 1937.

Western New York

Chairman: W. J. Atwell. Secretary: R. R. Humphrey. Members: 56.
Meetings: Cornell University, October 31, 1936.
Syracuse University, December 19, 1936.
University of Rochester Medical School, February 13, 1937.
University of Buffalo, May 22, 1937.

Wisconsin

Chairman: E. L. Sevringhaus. Secretary: M. R. Irwin. Members: 35.

PROCEEDINGS

Preliminary manuscripts, 47%; complete manuscripts, 53%.

Radical revision or non-acceptance of manuscripts is determined by three or more editors.

MEMBERSHIP

Members, March 31, 1936	1318	
Elected during year	60	
Honorary Members	8	
Total		1386
Resignations	12	
Deaths	11	
Arrears	9	
		32
Total Membership, March 31, 1937		1354

	1927	1937
Membership	851	1354

Subscriptions, March 31, 1937 544

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: Doctors S. R. Benedict, H. Brooks, R. Ellsworth, G. L. Hoffman, C. W. Hooper, C. G. Huber, F. M. Johns, E. O. Jordan, O. Klotz, M. Marriott, and P. G. Stiles.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT

April 1, 1936 to April 1, 1937

The officers of the Society express their profound appreciation of the superb technical help of Mr. H. G. Friedman in the investments of the Society. It is due largely to his help that the Society weathered so successfully the protracted financial depression.

The officers also wish to thank Mr. Leon Leighton, who also voluntarily advised us in the many legal questions concerning our mortgages.

RECEIPTS

Balance on Hand, April 1, 1936		$ 5,996.17
Income, 1936-37		
Dues	$ 5,569.75	
Reprints	2,854.51	
Excess Space	1,326.19	
Cuts	545.00	
Changes	39.02	
Subscriptions	3,318.24	
Back Numbers Sold	246.48	
Interest (Special accounts)	12.41	
Miscellaneous	18.03	
Total Annual Income		$13,929.63
Total Cash Available, April 1, 1936-April 1, 1937		$19,925.80

DISBURSEMENTS

Publication Cost of Proceedings:		
Printing	$ 6,730.12	
Reprints	2,848.09	
Cuts	698.74	
		$10,276.95
Administrative Expenses:		
Office Supplies, Postage, and Telephone	$ 1,023.74	
Salary	2,064.00	
Storage and Insurance	71.13	
*Miscellaneous	152.19	
		3,311.06
Total Annual Disbursements		$13,588.01
Cash Balance, April 1, 1937		6,337.79
Total		$19,925.80

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*Includes incorporation fees—$85.

Summary

Income (net)	$13,929.63
Disbursements (net)	13,588.01
Surplus	$ 341.62

Bills payable—none
Bills receivable—$714.42

Funds

Endowment Fund

April 1, 1936	$15,390.92	
Interest to April 1, 1937	614.97	
		$16,005.89
Invested in New York Title and Mortgage Co.	$ 6,000.00	
Title Guarantee and Trust Co.	2,500.00	
Lawyers Mortgage Co.	1,500.00	
R. R. Federal Savings and Loan	24.71	
Bowery Savings Bank	1,008.02	
Industrial Bonds	4,973.16	
(Present value $5,067.13)		$16,005.89

Surplus Fund

April 1, 1936	$ 9,693.72	
Interest to April 1, 1937	370.72	
		$10,064.44
Invested in Title Guarantee and Trust Co.	$ 2,900.00	
R. R. Federal Savings and Loan	3,103.87	
Bowery Savings Bank	887.10	
Industrial Bonds	3,173.47	
(Present value $3,345.00)		$10,064.44

Life Membership Fund

Invested in R. R. Federal Savings and Loan	$ 75.00

Auditors' Report

April 16, 1937.

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We are satisfied that the records of financial transactions are in good order and accurately kept.

(Signed) Alwin M. Pappenheimer
Homer W. Smith

MEMBERS' LIST

Honorary Members

Flexner, Simon	Rockefeller Institute
Howell, William H.	Johns Hopkins Univ.
Lee, F. S.	New York City
Lombard, Warren P.	Univ. of Michigan
Porter, William	Harvard Univ.
Richet, Charles	Paris, France
Von Muller, Friedrich	Munich, Germany
Wilson, Edmund B.	Columbia Univ.

Members

Abel, John J.	Johns Hopkins Univ.
Abramson, H. A.	Coll. Physicians and Surgeons, New York
Abt, Arthur F.	Northwestern Univ.
Adams, A. Elizabeth	Mount Holyoke Coll.
Adams, William E.	Univ. of Chicago
Addis, Thomas	Med., Stanford Univ.
Adolph, E. F.	Univ. of Rochester Med.
Adolph, W. H.	Peiping, China
Alexander, Harry L.	Washington Univ.
Allen, Bennet M.	Univ. of California, Los Angeles
Allen, Edgar	Yale Univ.
Allen, Ezra	N. Y. Homeopathic Med.
Allen, William F.	Univ. of Oregon
Alles, G. A.	Los Angeles, California
Alsberg, Carl L.	Stanford Univ.
Alt, Howard L.	Northwestern Univ. Med.
Althausen, T. L.	Univ. of California Med.
Altschule, M. D.	Beth Israel Hospital, Boston
Alvarez, Walter C.	Mayo Clinic
Alving, A. S.	Univ. of Chicago
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	Univ. of Tennessee Med.
Amoss, Harold L.	Rockefeller Institute
Andersen, Dorothy H.	Coll. of Physicians and Surgeons
Anderson, H. H.	Univ. of California Med.
Anderson, John E.	Univ. of Minnesota
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale Univ.
Anderson, William E.	Yale Univ.
Andrews, Edmund	Univ. of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd	Univ. of Illinois
Aronson, J. D.	Henry Phipps Institute
Asdell, S. A.	Cornell Univ.

Asher, Leon	Berne, Switzerland
Ashman, Richard	Louisiana State Univ. Medical Center
Asmundson, V. S.	Univ. of California
Atchley, D. W.	Presbyterian Hospital, New York City
Atwell, Wayne J.	Univ. of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis Univ.
Austin, J. Harold	Univ. of Pennsylvania
Avery, O. T.	Rockefeller Institute, N. Y. City
Avery, Roy C.	Vanderbilt Univ.
Aycock, W. L.	Harvard Med. School

Babkin, Boris P.	McGill Univ.
Bachem, Albert	Univ. of Illinois Medical Coll.
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Med.
Baitsell, George A.	Yale Univ.
Baker, Lillian E.	Rockefeller Institute
Bakwin, Harry	N. Y. Univ. and Bellevue Medical Coll.
Baldwin, Francis M.	Univ. of S. Calif.
Ball, G. H.	Univ. of California, Los Angeles
Ball, H. A.	San Diego, Calif.
Balls, A. K.	U. S. Dept. of Agriculture
Banta, A. M.	Brown Univ.
Banting, Frederick G.	Univ. of Toronto
Barach, Alvin L.	Coll. of Physicians and Surgeons
Barber, W. Howard	New York Univ. Med.
Barbour, Henry G.	Yale Univ.
Barer, Adelaide P.	State Univ. of Iowa
Barlow, O. W.	Albany, N. Y.
Barnett, George D.	Stanford Univ.
Barr, David P.	Washington Univ.
Barron, E. S. G.	Univ. of Chicago
Barth, L. G.	Columbia Univ.
Bartley, S. H.	Washington Univ.
Bass, Charles	Tulane Univ.
Bast, T. H.	Univ. of Wisconsin
Bates, R. W.	Carnegie Institution of Washington
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford Univ.
Bayne-Jones, S.	Yale Univ.
Bazett, H. C.	Univ. of Pennsylvania
Bean, John W.	Univ. of Michigan
Beard, H. H.	Louisiana State Univ. Medical Center
Beard, J. W.	Rockefeller Institute
Beard, P. J.	Stanford Univ.
Beck, Claude S.	Western Reserve Univ.
Becker, E. R.	Iowa State Coll.

Beckman, Harry	Marquette Univ. Med.
Beckwith, T. D.	Univ. of California, Los Angeles
Behre, Jeannette A.	Cornell Univ. Medical Coll.
Belding, David L.	Boston Univ.
Bellamy, Arthur W.	Univ. of California, Los Angeles
Berg, B. N.	Columbia Univ.
Berg, C. P.	State Univ. of Iowa
Berg, William N.	New York City
Bergeim, Olaf	Univ. of Illinois
Bergey, David H.	National Drug Co., Philadelphia
Bergmann, Max	Rockefeller Institute
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Bernthal, T. G.	Univ. of Michigan
Berry, George P.	Univ. of Rochester Med.
Beutner, R.	Hahnemann Medical College, Philadelphia
Bieter, Raymond N.	Univ. of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C.	American Med. Assn., Chicago
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	Geophysic Institute, Bergen, Norway
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	Univ. of California
Blackfan, K. D.	Harvard Med. School
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale Univ.
Blakeslee, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt Univ. Med.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., N. Y. City
Blau, Nathan F.	Cornell Univ. Medical Coll.
Blinks, L. R.	Stanford Univ.
Bliss, Sidney	Tulane Univ.
Bloch, Robert G.	Univ. of Chicago
Block, Richard J.	N. Y. State Psychiatric Institute
Bloom, William	Univ. of Chicago
Bloomfield, A. L.	Stanford Univ. Med.
Bloor, W. R.	Univ. of Rochester
Blount, R. F.	Univ. of Minnesota
Blum, Harold F.	Univ. of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bock, Joseph C.	Marquette Univ.
Bodansky, A.	Hospital for Joint Diseases, N. Y. City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodine, J. H.	State Univ. of Iowa
Bodo, Richard	N. Y. Univ. and Bellevue Medical Coll.
Bogen, Emil	Olive View, Calif.
Boissevain, Charles H.	Colorado College
Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	Univ. of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Presbyterian Hospital, N. Y. City
Borsook, Henry	California Institute of Technology

Bowen, B. D.	Buffalo General Hospital
Boyd, Eldon M.	Queens University, Canada
Boyd, Theo. E.	Loyola Univ.
Boyden, E. A.	Univ. of Minnesota Med.
Bradford, William L.	Univ. of Rochester
Bradley, H. C.	Univ. of Wisconsin
Brand, Erwin	N. Y. State Psychiatric Institute
Branham, Sara E.	National Inst. of Health, Washington
Brewer, Robert K.	Syracuse Univ.
Briggs, A. P.	Univ. of Georgia
Brodie, Maurice	Providence Hospital, Detroit, Mich.
Bronfenbrenner, J.	Washington Univ.
Bronk, D. W.	Univ. of Pennsylvania
Brooks, Clyde	Louisiana State Univ. Medical Center
Brooks, Matilda M.	Univ. of California
Brooks, S. C.	Univ. of California
Broun, G. O.	St. Louis Univ. Med.
Brown, E. D.	Univ. of Minnesota
Brown, J. Howard	Johns Hopkins Univ.
Brown, John B.	Ohio State Univ.
Brown, L. A.	Transylvania Coll., Lexington, Ky.
Brown, Rachel	N. Y. State Dept. of Health
Brown, Wade H.	Rockefeller Institute, Princeton
Bruger, Maurice	N. Y. Post-Graduate Med.
Brunschwig, Alexander	Univ. of Chicago
Buchanan, Robert E.	Iowa State Coll.
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Buell, Mary V.	Johns Hopkins Univ.
Bulger, H. A.	Washington Univ.
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	Univ. of Wisconsin
Bunting, R. W.	Univ. of Michigan
Burch, John C.	Vanderbilt Univ. Med.
Burget, G. E.	Univ. of Oregon Med.
Burky, Earl L.	Johns Hopkins Hospital
Burns, Robert K., Jr.	Univ. of Rochester
Burr, George O.	Univ. of Minnesota
Burr, Harold S.	Yale Univ.
Burrows, M. T.	Pasadena, Calif.
Butt, E. M.	Univ. of Southern California
Butts, Joseph S.	Univ. of Southern California
Byerly, T. C.	U. S. Animal Exp. Farm, Beltsville, Md.
Byrne, Joseph	Fordham Univ.

Calkins, Gary N.	Columbia Univ.
Cameron, A. T.	Univ. of Manitoba
Cannan, Robert K.	N. Y. Univ. and Bellevue Medical Coll.
Cannon, Paul R.	Univ. of Chicago
Cannon, Walter B.	Harvard Med. School
Cantarow, Abraham	Jefferson Medical Coll.
Carey, E. J.	Marquette Univ.

Carlson, A. J.	Univ. of Chicago
Carmichael, E. B.	Univ. of Alabama Med.
Carruthers, Albert	Peiping Union Medical Coll.
Carter, Edward P.	Johns Hopkins Hospital
Casey, Albert E.	Univ. of Virginia
Cash, James R.	Univ. of Virginia
Casida, L. E.	Univ. of Wisconsin
Cattell, McKeen	Cornell Univ. Medical Coll.
Caulfeild, A. H. W.	Univ. of Toronto
Cecil, R. L.	Cornell Univ. Medical Coll.
Cerecedo, L. R.	Fordham Univ.
Chace, Arthur F.	N. Y. Post-Graduate Medical Coll.
Chaikoff, I. L.	Univ. of California
Chambers, Robert	New York Univ.
Chambers, William H.	Cornell Univ. Medical Coll.
Chargaff, Erwin	Coll. of Physicians and Surgeons
Chang, Hsi Chun	Peiping Union Medical Coll.
Chang, Hsiao-Chien	Peiping Union Medical Coll.
Charipper, H. A.	New York Univ.
Cheer, S. N.	Central Hospital, Nanking, China
Chen, Graham M.	Peiping Union Medical Coll.
Chen, K. K.	Eli Lilly and Co., Indianapolis
Chen, T. T.	Peiping Union Medical Coll.
Cheney, R. H.	Long Island Univ.
Chidester, F. E.	Syracuse, N. Y.
Child, C. M.	Stanford Univ.
Chittenden, R. H.	Yale Univ.
Christensen, K.	St. Louis Univ.
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	Univ. of Michigan
Chu, F. T.	Peiping Union Medical Coll.
Clark, A. J.	University of Edinburgh, Scotland
Clark, Ada R.	Coll. of Physicians and Surgeons
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	Univ. of Wisconsin
Clarke, Hans T.	Coll. of Physicians and Surgeons
Claude, A.	Rockefeller Inst.
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clawson, Benjamin J.	Univ. of Minnesota
Clifton, Charles E.	Stanford Univ.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Oradell, N. J.
Coggeshall, L. T.	Rockefeller Foundation
Coghill, G. E.	Gainesville, Fla.
Cohen, Barnett	Johns Hopkins Med.
Cohen, Martin	N. Y. Post-Graduate Med.
Cohen, Milton B.	St. Alexis Hospital, Cleveland
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, Arthur G.	Univ. of Illinois Med.
Cole, E. C.	Williams Coll.

Cole, Harold H.	Univ. of California, Davis
Cole, L. J.	Univ. of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers Univ.
Coleman, Warren	New York Univ. Medical Coll.
Collens, William S.	Brooklyn, N. Y.
Collett, Mary E.	Western Reserve Univ.
Collier, William D.	St. Elizabeth's Hospital, Youngstown, O.
Collins, H. H.	Crafton, Pa.
Collip, J. B.	McGill Univ.
Conklin, E. G.	Princeton Univ.
Connor, Charles L.	Univ. of California Med.
Cook, Donald H.	School of Tropical Medicine, San Juan, P. R.
Cooke, J. V.	Washington Univ.
Coombs, Helen C.	N. Y. Homeopathic Medical Coll.
Cope, O. M.	N. Y. Homeopathic Med.
Copenhaver, W. M.	Columbia Univ.
Corey, E. L.	Univ. of Va.
Cori, Carl F.	Washington Univ.
Corley, Ralph C.	Purdue Univ.
Corner, George W.	Univ. of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
CoTui	N. Y. Univ. Medical Coll.
Coulter, Calvin B.	Columbia Univ.
Cowdry, E. V.	Washington Univ.
Cowgill, George R.	Yale Univ.
Cowie, D. M.	Univ. of Michigan
Cox, Herald R.	U. S. Public Health Inst., Hamilton, Mont.
Cox, Warren M., Jr.	Mead Johnson Co.
Craig, C. F.	Tulane Univ.
Cram, Eloise B.	Nat. Inst. of Health, Washington
Crampton, C. Ward	N. Y. Post-Graduate Med.
Crandall, L. A., Jr.	Northwestern Univ.
Crile, George W.	Western Reserve Univ.
Crittenden, Phoebe J.	George Washington Univ.
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Csonka, F. A.	U. S. Dept. of Agriculture, Washington, D. C.
Cullen, Glenn E.	Children's Hospital, Cincinnati, O.
Cummins, Harold	Tulane Univ.
Cunningham, R. S.	Vanderbilt Univ.
Curtis, G. M.	Ohio State Univ.
Curtis, Maynie R.	Columbia Univ.
Cushing, Harvey W.	Yale Univ.
Cutler, Elliott C.	Peter Bent Brigham Hospital, Boston
Dack, Gail M.	Univ. of Chicago
Dakin, H. D.	Ossining, N. Y.
D'Amour, F. E.	Univ. of Denver
Danforth, Charles H.	Stanford Univ.
Daniel, J. Frank	Univ. of California

Daniels, Amy L.	Univ. of Iowa
Danzer, Charles S.	N. Y. Homeopathic Medical Coll.
Dautrebande, Lucien	Univ. of Liege, Belgium
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davenport, H. A.	Northwestern Univ. Med.
Davis, D. J.	Wilmette, Illinois
Davis, M. E.	Univ. of Chicago
Dawson, James A.	Coll. of the City of N. Y.
Dawson, M. H.	Columbia Univ.
Dawson, W. T.	Univ. of Texas Med.
Day, A. A.	Northwestern Univ. Med.
Decherd, George M.	Louisiana State Univ.
DeEds, Floyd	Stanford Univ. Med.
DeGraff, A. C.	N. Y. Univ. Med.
Dennis, E. W.	Amer. Univ. of Beirut
DeRenyi, G. S.	Univ. of Pennsylvania
DeSavitsch, Eugene	Univ. of Chicago
Derick, C. L.	Peter Bent Brigham Hospital, Boston
Detwiler, S. R.	Columbia Univ.
Deuel, Harry J., Jr.	Univ. of S. Calif. Med.
Dick, George F.	Univ. of Chicago
Dickson, E. C.	Stanford Univ. Med.
Dieckmann, W. J.	Univ. of Chicago
Dienes, Louis	Massachusetts General Hospital, Boston
Dieuaide, Francis R.	Peiping Union Medical Coll.
Doan, Charles A.	Ohio State Univ.
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford Univ.
Doisy, Edward A.	St. Louis Univ.
Dolley, W. L., Jr.	Univ. of Buffalo
Dominguez, R.	St. Luke's Hospital, Cleveland
Domm, L. V.	Univ. of Chicago
Donaldson, H. H.	Wistar Institute, Philadelphia
Donaldson, J. C.	Univ. of Pittsburgh
Dooley, M. S.	Syracuse Univ.
Doull, J. A.	Western Reserve Univ.
Downes, Helen R.	Memorial Hospital, N. Y. City
Drabkin, D. L.	Univ. of Pennsylvania
Dragstedt, Carl A.	Northwestern Univ.
Dragstedt, Lester R.	Univ. of Chicago
Draper, George W.	Columbia Univ.
Draper, William B.	Univ. of Colorado
Dresbach, M.	Boston, Mass.
Drury, D. R.	Univ. of S. Calif. Med.
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell Univ. Medical Coll.
DuBois, F. S.	Univ. of Alabama
Duggar, B. M.	Univ. of Wisconsin
Dukes, H. H.	Cornell Univ.
Dunn, Leslie C.	Columbia Univ.
Dunn, Max	Univ. of California, Los Angeles

Duran-Reynals, F.	Rockefeller Institute
Dusser de Barenne, J. G.	Yale Univ.
Dutcher, R. Adams	Pennsylvania State Coll.
Duval, C. W.	Tulane Univ.
Du Vigneaud, Vincent	George Washington Univ. Med.
Dye, Joseph A.	Cornell Univ. Med.
Dyer, Helen M.	George Washington Univ. Med.

Earle, Wilton R.	National Inst. of Health, Washington
Eastman, N. J.	Johns Hopkins Univ.
Eberson, Frederick	San Francisco, Calif.
Ecker, E. E.	Western Reserve Univ.
Eckstein, Henry C.	Univ. of Michigan
Eddy, Walter H.	Columbia Univ.
Edmunds, C. W.	Univ. of Michigan
Edwards, D. J.	Cornell Univ. Medical Coll.
Edwards, John G.	Univ. of Buffalo
Eggston, Andrew A.	N. Y. Manhattan Eye, Ear Hospital
Ellis, Max M.	Univ. of Missouri
Elsberg, Charles A.	Neurological Institute, N. Y. City
Elser, W. J.	Cornell Univ. Medical Coll.
Emerson, G. A.	West Virginia Univ. Med.
Emery, F. E.	Univ. of Buffalo
Emge, L. A.	Stanford Univ.
Engle, E. T.	Columbia Univ.
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erlanger, Joseph	Washington Univ.
Ernstene, Arthur C.	Cleveland Clinic
Essex, Hiram E.	Mayo Clinic
Evans, Alice C.	National Inst. of Health, Washington
Evans, Herbert M.	Univ. of California
Everett, M. R.	Univ. of Oklahoma Med.
Ewing, James	Cornell Univ. Medical Coll.
Eyster, J. A. E.	Univ. of Wisconsin

Faber, Harold K.	Stanford Univ. Med.
Fahr, George	Univ. of Minnesota
Falk, I. S.	Washington, D. C.
Falk, K. George	N. Y. Univ. Medical Coll.
Falls, Frederick H.	Univ. of Illinois
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Farmer, Chester	Northwestern Univ.
Faust, Ernest C.	Tulane Univ.
Feil, Harold	Western Reserve Univ.
Feng, T. P.	Peiping Union Med.
Fenn, Wallace O.	Univ. of Rochester Med.
Ferguson, John H.	University of Alabama Med. School
Ferraro, Armando	N. Y. State Psychiatric Institute
Ferry, R. M.	Harvard Univ.
Field, John, II.	Stanford Univ.
Figge, F. H. J.	Univ. of Maryland Med.

Fine, M. S.	Battle Creek, Mich.
Firor, W. M.	Johns Hopkins Univ.
Fischer, Albert	Kopenhagen, Denmark
Fischer, E.	Medical Coll. of Va.
Fischer, Martin H.	Univ. of Cincinnati
Fishberg, Ella H.	Beth Israel Hospital, N. Y. City
Fitch, C. P.	Univ. of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
Fitzgerald, J. G.	Univ. of Toronto
Fleisher, Moyer S.	St. Louis Univ.
Flinn, Frederick B.	Columbia Univ.
Florence, Laura	N. Y. Homeopathic Med.
Fluhmann, Charles F.	Stanford Univ. Med.
Forbes, Henry	Milton, Mass.
Forkner, Claude E.	Peiping Union Medical Coll.
Fortuyn, A. B. D.	Peiping Union Medical Coll.
Foster, Goodwin L.	Columbia Univ.
Fowler, Willis M.	State Univ. of Iowa
Fox, D. L.	Scripps Institution, LaJolla, Calif.
Francis, Thomas, Jr.	Rockefeller Institute
Frank, Robert T.	Mt. Sinai Hospital
Frazier, Chester N.	Peiping Union Medical Coll.
Freedlander, S. O.	Western Reserve Univ.
Freedman, Louis	New York City
Freeman, R. G., Jr.	Columbia Univ.
Freudenberger, C. B.	Univ. of Utah
Freund, Jules	Cornell Univ. Medical Coll.
Fridericia, L. S.	Univ. of Copenhagen, Denmark
Friedemann, T. E.	Univ. of Chicago
Friedman, M. H.	Univ. of Pennsylvania
Frobisher, Martin, Jr.	Baltimore, Md.
Fry, Henry J.	Cornell Medical Coll.
Fulton, John F.	Yale Med.
Funk, Casimir	Paris, France
Furth, Jacob	Cornell Univ. Medical Coll.
Gaebler, O. H.	Henry Ford Hospital, Detroit
Gage, I. M.	Tulane Univ.
Gager, C. Stuart	Brooklyn Botanic Garden, N. Y.
Gallagher, T. F.	Univ. of Chicago
Gamble, James L.	Harvard Univ.
Garbat, Abraham L.	Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.	Saranac Lab. Tuberculosis
Gardner, William U.	Yale Univ.
Garrey, Walter E.	Vanderbilt Univ.
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	Rockefeller Institute
Gaunt, Robert	New York Univ.
Gay, F. P.	Columbia Univ.
Geiling, E. M. K.	Univ. of Chicago
Geist, Samuel H.	Mt. Sinai Hospital, N. Y. City

Gellhorn, Ernst	Univ. of Illinois Medical Coll.
Gengerelli, J. A.	Univ. of California, Los Angeles
Gerard, R. W.	Univ. of Chicago
Gerstenberger, H. J.	Western Reserve Univ.
Gesell, Robert A.	Univ. of Michigan
Gettler, A. O.	N. Y. Univ. Medical Coll.
Geyelin, H. R.	Columbia Univ.
Gibbs, O. S.	Univ. of Tenn.
Gies, William J.	Columbia Univ.
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Graham, Helen T.	Washington Univ.
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